IN SITU AND EX SITU BIOREMEDIATION OF RADIONUCLIDES CONTAMINATED SOILS AT NUCLEAR AND NORM SITES

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Chapter 9   *In situ* and *ex situ* bioremediation of radionuclides contaminated soils at nuclear and NORM sites

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Abstract

Radioactive contamination of the environment is a global problem arising from generating nuclear power nuclear accidents, testing nuclear weapons and from disposing of nuclear waste. Microorganisms potentially can solubilize and immobilize a wide range of naturally occurring radionuclides, actinides, and fission products. Their ability to do so encompasses several mechanisms, such as oxidation-reduction reactions, the production of sequestering agents, bioaccumulation, and biocrystallization. Presently, such microbial processes are exploited to remediate radionuclide-contaminated environments. In this chapter we briefly review the key microbial processes and the mechanisms underlying the biotransformation of radionuclides of concern, and consider their potential application for *in situ* or *ex situ* bioremediation of radionuclide- contaminated soils, sediments, and wastes.

20.1   Introduction

The contamination of soils with radionuclides due to the many phases of the nuclear fuel cycle is a major problem. These activities include mining and milling the uranium, constructing and operating facilities for fabricating the nuclear fuel, and operating nuclear-power plants, as well as nuclear accidents, disposal of nuclear wastes (low- and intermediate-level waste (LILW), transuranic (TRU) wastes), including depleted uranium, reprocessing the

spent nuclear fuel, and producing and testing nuclear weapons. Added to these problems are those inherent in phosphate mining, drilling for oil and gas, and disposing of their wastes. The radionuclides of concern from these activities include the actinides (Th, U, Np, Pu, Am), fission products (^{137}Cs, ^{90}Sr, ^{99}Tc, ^{129}I, ^{79}Se), activation products (^{60}Co, ^{63}Ni, ^{14}C, ^{3}H) and the naturally occurring radionuclides Th, U, and ^{226}Ra. These radionuclides are either α, β, and/or γ emitters, most with very long half-lives and high radiotoxicity. Remediating the contaminated soils requires removing these radionuclides and returning the clean soil to normal use, or if, appropriate immobilizing the radionuclides *in-situ* so that they are stable and do not move into the environment. The latter process involves long-term monitoring and management of such sites that may or may not be accessible for public use, or until they pose no hazard to public health. In most cases, remediating large volumes of soils, albeit contaminated with low levels of radionuclides, is problematic in terms of the sheer volume of the waste to be dealt with, and the lack of appropriate technology to remove the radionuclides.

Bioremediation is the transformation or degradation of contaminants into non-hazardous or less hazardous chemical forms. Bioremediation is an attractive and promising green technology. Bacteria commonly are used, but other organisms, such as fungi, algae, or plants also have been employed. Unlike organic compounds, radionuclides and toxic metals cannot be converted into innocuous compounds but must be removed or stabilized. Understanding the role of microbes in the environmental fate of radionuclides and the underlying mechanisms in microbe–radionuclide interactions is of great importance for developing effective methods of bioremediation for contaminated environments and biotechnology for treatment and managing hazardous wastes.

Microbes play a major role in the biogeochemical cycling of metals and radionuclides. The electron-transfer reactions are central to energy and the cycling of various elements in

the environment. Microbial activity can affect the stability and the mobility of radionuclides in most soils. Depending on the radionuclide and the site-specific soil conditions, radionuclides can be solubilized or immobilized by the direct enzymatic- or indirect non-enzymatic-actions of microorganisms. Several radionuclides and metals are redox-active and can be oxidized and reduced by microorganisms. Microbial reduction of certain radionuclides alters their solubility, thereby decreasing their mobility, bioavailability, and toxicity. Radionuclides initially present in soil in soluble form may be converted to an insoluble form or vice versa by the actions of microorganisms. Bioremediation methods include natural attenuation, biostimulation, and bioaugmentation. Natural attenuation depends on the intrinsic bioremediation potential, and involves little or no human intervention. In biostimulation, electron donors, electron acceptors, or trace element nutrients are injected into the soil to stimulate the growth and activity of indigenous microorganisms. Bioaugmentation involves deliberately adding microorganisms with the desired catabolic traits to accelerate metabolic conversions. These processes are exploited in designing in-situ- or ex-situ- bioremediation strategies involving the selective dissolution or immobilization of radionuclides.

20.2 Radionuclides in contaminated soils, sediments, and wastes

Contamination of the soils with radionuclides arises primarily from several sources: (i) The front- and back-end of the nuclear fuel cycle (uranium mining and milling operations, nuclear-fuel fabrication facilities, nuclear-power-plant operation, controlled release of gaseous radioactive components into the atmosphere, and reprocessing spent nuclear fuels); (ii) the extraction of Th and rare-earth elements from monazite ore; (iii) the operation of nuclear-weapons production facilities; (iv) low-, intermediate-, and high-level radioactive waste disposal sites; (v) the production of natural gas and oil, (vi) the combustion of fossil

fuels; (vii) mining phosphate and applying fertilizer; (viii) accidents at nuclear power plants; and, (viii) dirty bombs. The radionuclides in contaminated soils, sludge, and wastes may exist in various chemical forms, such as elemental, oxide, co-precipitates, ionic-, inorganic-, and organic-complexes that regulate their mobility and stability in the environment. The mode of effectively remediating contaminated soils primarily is dictated by the nature of the chemical speciation, and the mineralogical association of the radionuclides with the solid phases.

20.2.1 Naturally Occurring Radionuclides. Naturally Occurring Radionuclide Materials (NORMs) are those natural radioactive elements present in very low concentrations in the earth's crust. They include the long-lived radionuclides, such as uranium, thorium, radium and its decay product radon, and, in some instances, trace amounts of the short-lived polonium-210 (half-life of 138 days). Elevated concentrations of radionuclides often are found in certain sedimentary- and igneous-rocks and ores. Industrial activities that exploit the natural resources may lead to increased concentrations of radionuclides, often referred to as technologically enhanced naturally occurring radioactive material (TE-NORM), and thus results in products, by-products, residues, and wastes containing radionuclides. Such activities include the mining and processing of ores, the production of natural gas and oil, the combustion of fossil fuels, or the production of coal ash from burning coal in power plants.

20.2.1.1   NORM in the exploration of oil and gas. Enhanced concentrations of the radium-226 and 228 accumulate in oil-field pits, tanks, lagoons, and in sludge. Radon gas in the natural-gas streams concentrate as NORM in gas-processing activities. Radon decays to lead-210, then to bismuth-210, polonium-210, and finally stabilizes as lead-206. Radon-decay elements occur as a shiny film on the inner surface of the inlet lines, treating units, pumps, and valves associated with the processing systems for propylene, ethane, and propane. The NORM waste may occur in a crystalline form, in carbonate matrix, or as white or a brown solid scalesv or a thick sludge as solid, dry flaky substances.

20.2.1.2 *NORM from phosphate mining*. Phosphate fertilizers are obtained from phosphorites of sedimentary or magmatic origin. Sedimentary phosphate rock usually is strip-mined and contains high concentrations of ^{238}U (800-5200 Bq/kg), ^{230}Th (200-16,000 Bq/kg), ^{232}Th (5-170 Bq/kg), and ^{226}Ra (25-900 Bq/kg). Apatite $Ca_2[(PO4)_3(OH),F,Cl)]$, the predominant mineral also contains trace amounts of ^{210}Po (Merkel and Hoyer, 2012). The mining and processing of phosphate fertilizers contaminate the surrounding soil and the application of the fertilizer over time tends to increase the concentration of radionuclides in agricultural soils, and thus transfer radionuclides through the food chain.

20.2.1.3 *Uranium mining and mill tailing*. Traditional uranium mining generates fine sandy tailings that contain virtually all the naturally occurring radioactive elements found in uranium ore. These are collected in engineered tailings dams and finally covered with a layer of clay and rock to inhibit the leakage of radon gas and ensure their long-term stability. Over the short term, the tailings material often is covered with water. After a few months, the tailings material contains about 75% of the radioactivity of the original ore; these are not classified as radioactive wastes. Uranium tailings are waste by-product materials left over from processing uranium-bearing ore. Uranium mill tailings also contain heavy metals, such as lead and arsenic. Large mounds of uranium-mill tailings have been left at many old mining sites, and so remain a potential radiological- and toxic-metal-hazard mainly due to the solubilization of ^{226}Ra, As, and Pb from ore minerals.

20.2.1.4 *Weapons production and testing.* Surface- and subsurface-contamination of actinides and fission products due to nuclear-fuel processing, weapons production, and weapons testing is a huge problem. The contamination of soils and sediments with actinides, such as Th, U, Pu, Np, Am, and with the fission products ^{99}Tc, ^{90}Sr, ^{137}Cs, ^{129}I was reported. Such sites occur in the USA (Bikini Atoll, the Rocky Flats, Colorado, Y-12 Plant, Oak Ridge, Tennessee, Fernald site, Ohio, Nevada Test Site, Nevada, Hanford,

Washington, among others), the Myak facility and others in Russia, Sellafield in the UK, and also in Germany, France, Belgium, Belarus, and the Ukraine.

20.2.1.5 **Accidents at *nuclear-power plants.*** Widespread radioactive contamination of soil was caused by the accident at Chernobyl, and the nuclear disaster at the Fukushima Daiichi plant. The explosion and fire at Chernobyl threw hot particles of the nuclear fuel and fission products into the air. Approximately 100,000 km² of land was contaminated significantly by the fallout. The primary radionuclides of concern are the fission products, ^{137}Cs and ^{90}Sr, and minor amounts of the TRU elements Np, $^{239, 240}$Pu, ^{241}Am, and others embedded in a uranium-oxide matrix. ^{137}Cs contamination at these sites is extensive and covers a large area with a range of concentrations, while actinide contamination is more localized to the accident area. The ^{137}Cs levels are very high in certain locales that it prevents re-inhabitation at both Chernobyl and Fukushima. ^{137}Cs and ^{90}Sr will continue to be of greatest concern for several decades, while Pu isotopes and ^{241}Am will be of concern for hundreds and thousands of years, particularly in the immediate vicinity of the accident site.

20.2.1.6 ***Nuclear Wastes.*** Nuclear wastes are classified as Low-level wastes (LLWs), Intermediate-level wastes (ILWs), Transuranic wastes (TRUs), and High-level wastes (HLWs).

20.2.1.6.1 ***Low-level waste (LLW)***. LLW is generated from hospitals, industry, and from the operation of nuclear power plants. It comprises paper, rags, tools, clothing, and filters that contain small amounts of mostly short-lived radionuclides. It does not require shielding during handling and transport and is suitable for burial in shallow land. To reduce its volume, it often is compacted or incinerated before disposal. It comprises about 90% of the volume of waste, but only 1% of the radioactivity.

20.2.1.6.2 *Intermediate-level waste (ILW)*. ILW contains higher amounts of radioactivity, and, in some cases, requires shielding. It includes resins, chemical sludge, metal reactor nuclear-fuel cladding, and contaminated materials from decommissioning reactors. It may be solidified in concrete or bitumen for disposal. As a general rule, long-lived waste from fuel and fuel reprocessing is disposed of in a geological repository.

20.2.1.6.3 *Transuranic (TRU) waste*. TRU waste as defined by U.S. regulations is, without regard to its form or origin, a waste that is contaminated with alpha-emitting transuranic radionuclides with half-lives greater than 20 years and concentrations greater than 100 nCi/g (3.7 MBq/kg), excluding high-level waste. Elements that have an atomic number greater than uranium are called transuranics "beyond uranium". In the United States, it arises mainly from producing weapons, , and consists of clothing, tools, rags, residues, debris, and other items contaminated with small amounts of radioactive elements, mainly plutonium. Under U.S. law, transuranic waste is categorized further into "contact-handled" (CH) and "remote-handled" (RH) on the basis of the radiation dose measured at the surface of the waste container. CH TRU waste has a surface dose rate not greater than 200 Roentgen equivalent person per hour (two millisievert/hr (2 mSv/h)), whereas RH TRU waste has a surface dose rate of 200 Röntgen equivalent person per hour (2 mSv/h) or greater. CH TRU waste does not have the very high radioactivity of high-level waste, nor high heat-generation, but RH TRU waste can be highly radioactive, with surface dose rates up to 1,000,000 Röntgen equivalent person per hour (10,000 mSv/h). The United States currently disposes of TRU waste generated from weapons-production facilities at the Waste Isolation Pilot Plant (WIPP), in deep underground geological salt formations located in Carlsbad, New Mexico.

20.2.1.6.4 *High-level waste (HLW).* HLW arises from the 'burning' of uranium fuel in a nuclear reactor; it contains the fission products and transuranic elements generated in the reactor's core. It is highly radioactive and hot, so requires cooling and shielding. HLW

accounts for over 95% of the total radioactivity produced in generating electricity. Typically, HLW consists of spent fuel rods and separated waste from reprocessing the used fuel that normally is vitrified.

20.3   Characterization of radionuclides in contaminated soils and wastes.

The chemical speciation and mineralogical association of radionuclides present in soils or wastes regulate their mobility and bioavailability. The radionuclides and toxic metals most commonly found in soils and wastes are americium, cadmium, cesium, chromium, cobalt, copper, lead, manganese, nickel, plutonium, technetium, thorium, strontium, uranium, and zinc. Typically, these contaminants are present in various forms, such as elemental, carbonate complexes, oxides, co-precipitates (metals co-precipitated with iron and manganese oxides), natural minerals, and soluble- and insoluble-organic complexes. In soils and wastes they may be present initially as soluble forms, or they may be formed after disposal by chemical- and microbiological-processes (weathering). Detailed information on the types and characteristics of the contaminants in soils and wastes is a prerequisite for selecting or developing appropriate bioremediation technology. The data needed include the chemical identity, concentration, speciation, and the mineralogical association of radionuclides and toxic metals. Chemical speciation is an important factor influencing the behaviors of metal ions in the environment. Chemical speciation refers to the chemical form in which a molecule or ion exists, whether it is a monomer or polymer, a dissolved aquo- or hydroxo-complex, or an insoluble oxide, and the like. Depending on the nature of the problem being studied, there can be multiple levels of information on speciation. These include (i) the identity of the element, (ii) its physical state, (ii) its oxidation state, (iv) its molecular chemical formula, and, (v) its detailed molecular structure. A variety of approaches can be used for assessing chemical speciation. Techniques such as solubility measurements, potentiometry, liquid-liquid

partitioning, dialysis, chromatography, and optical spectroscopy have been employed for some time, and are considered classical methods. More recently, several more advanced spectroscopic techniques have been applied, including photoacoustic- and thermal-lensing, laser-induced fluorescence, magnetic resonance, and x-ray absorption spectroscopies. Each has its own particular sensitivity, specificity, cost, and benefit. Some methods can be used only for solid samples, whereas others can be used for both solid- and liquid-systems. In general, spectroscopic techniques, for instance, nuclear magnetic resonance (NMR) and x-ray absorption fine structure (XAFS) spectroscopy that yield detailed molecular structural information require relatively large concentrations of the target species (10^{-3} M), whereas techniques for obtaining only elemental composition, such as neutron activation analysis, can detect low concentrations (10^{-12} M).

The oxidation states of the redox active radionuclides and the presence of the radionuclides as soluble or associated with carbonate, iron and manganese oxide, organic-, silicate-, and inert-fractions provide information on the extent of the application of a particular *in-situ and ex-situ* remediation technology. For example, the radionuclides that are strongly bound to the soil matrix, such as the organic-, silicate-, and inert-fractions are far more difficult to remove and usually process is very expensive.

20.4   Mechanisms of biotransformation of radionuclides

The activities of microbes are influenced by the presence of electron donors and acceptors. Microorganisms use oxygen as an electron acceptor under aerobic conditions, while, in the absence of oxygen, they can use nitrates, sulfates, metals, and carbon dioxide as alternate electron acceptors. The form of the metal (e.g., elemental, oxide, sulfide, ionic, inorganic complex, organic complex, co-precipitate), the availability of electron donors, nutrients (nitrogen, phosphorus), the presence of electron acceptors (Fe^{3+}, Mn^{4+}, NO_3^-, SO_4^{2-}, organic

compounds), and the environmental factors (pH, Eh, temperature, moisture) affect the type, rate, and extent of microbial activity, and hence, the transformation of radionuclides at contaminated sites. Oxidizing- and reducing-conditions influence the mobilization and immobilization of radionuclides and toxic metals. In an anaerobic environment, certain metals and radionuclides are reduced enzymatically from a higher oxidation state to lower one, so affecting their solubility and bioavailability. For example, the reduction of $Fe^{3+} \rightarrow Fe^{2+}$ increases its solubility, while reducing $Cr^{6+} \rightarrow Cr^{3+}$ or $U^{6+} \rightarrow U^{4+}$ decreases their solubility. Many organic compounds form stable complexes with heavy metals and radionuclides, so increasing their solubilization and leaching. Likewise, microbial metabolites and waste-degradation products can affect the solubility of heavy metals and radionuclides. Biodegradation of the metal-, radionuclide-organic complexes may engender the precipitation of the metal or radionuclide, and so retard its mobility.

The mechanisms of microbial transformations of radionuclides are reviewed extensively elsewhere (Mackasckie, 1991; Lovely, 1993; Lloyd and Lovely, 2001; Francis, 1990; 2012). Briefly, the biotransformation of radionuclides includes one or more of the following mechanisms: (i) The oxidation-reduction of metals that affects their valence state and solubility; (ii) changes in pH that affect the ionic state of the metals and their solubility; (iii) solubilization, and leaching of elements by microbial metabolites and decomposition products, such as organic acid metabolites or the production of specific sequestering agents; (iv) volatilization due to alkylation reactions (biomethylation); (v) immobilization leading to the formation of stable minerals or bioaccumulation by microbial biomass and biopolymers; (vi) biotransformation of metal/radionuclide organic complexes, and, (vi) the remobilization of biosorbed or coprecipitated radionuclides due to mineralization. Of particular interest to the potential application to bioremediation are the oxidation-reduction reactions, solubilization, leaching, and the biodegradation of radionuclide-organic complexes.

20.3.1 Dissolution of radionuclides and metals. The direct and indirect actions of microorganisms occasion the dissolution of radionuclides and toxic metals. Direct action involves the dissolution of metals due to oxidation-reduction reactions. Indirect action entails dissolution due to the production of mineral acids, organic-acid metabolites, and oxidizing agents, as well as by lowering the pH of the medium.

20.3.1.1   Direct action. Many microorganisms solubilize metals by (i) oxidation (increase in valence state) or (ii) reduction (decrease in valence state). Direct action involves enzymatic reductive dissolution of the metal from a higher to a lower oxidation state, wherein the metal is used as the terminal electron acceptor. Microbial reduction and dissolution of iron and manganese oxides under anaerobic conditions has been studied extensively. For the most part, these redox reactions involve actively metabolizing bacteria. Reduction of metal ions also can occur passively when metals bind to reactive sites on the surface of, or within, microbial cells.

(i) Oxidative dissolution: Much of the information on the dissolution of metals deals with the oxidation of inorganic compounds, primarily from ore-leaching by the autotrophic microorganisms *Thiobacillus ferrooxidans* and *T. thiooxidans*. The chemical- and biochemical-mechanisms involved in microbial leaching or bio-mining of metals have been extensively studied and are used commercially for extracting copper and uranium from ores. The role of *T. ferrooxidans* in extracting uranium from ore results from indirect- and direct-actions. The indirect mechanism is confined to its generating the oxidizing agent, ferric sulfate, and the solvent sulfuric acid and to the involvement of Fe^{2+}/Fe^{3+} in mediating the oxidation of insoluble uranium oxide in a cyclical reaction to yield the soluble uranyl ion $UO_2 + Fe^{3+} \rightarrow UO_2^{2+} + Fe^{2+}$; in contrast, direct action involves the oxidation of $UO_2 \rightarrow UO_2^{2+}$ without using the Fe^{2+}/Fe^{3+} complex as the chemical electron-carrier.

(ii) Reductive dissolution: Reductive dissolution of metals from a higher to a lower oxidation state due to enzymatic action by facultative- and strict anaerobic-microorganisms has attracted much attention because of its potential application in the in-situ bioremediation of contaminants in the subsurface. Microbially-mediated metal reductions play an important role in the biogeochemical cycles of metals. Using microbially-mediated redox changes in metals for treating wastes and environmental remediation is attractive because the process can be carried out under mild conditions, does not produce hazardous secondary wastes, and potentially is applicable to a variety of waste forms. Examples of the enzymatic reductive dissolution of metals include the reduction of insoluble iron (Fe^{3+}) and manganese (Mn^{4+}) to their soluble forms Fe^{2+} and Mn^{2+}. Metal reduction can be mediated by actively metabolizing bacteria, or passively, wherein the metal is reduced when it interacts with binding sites both inside and outside the cell. These metals serve as alternate electron acceptors in the absence of oxygen for anaerobic respiration, or as an electron sink for the re-oxidation of reducing equivalents. Bioreduction catalyzed by several microorganisms has been utilized in solubilizing Mn(IV) and Fe(III), and in extracting metals from ores.

20.3.1.2 *Indirect Action.* The indirect dissolution of metals is due to the production of mineral acid from the oxidation of sulfide minerals by autotrophic bacteria, organic acids from the metabolism of organic compounds by heterotrophic bacteria, and the elaboration of metal-sequestering agents, such as siderophores. The mechanisms of bioleaching of metals from minerals by autotrophic microbes have been extensively investigated. An increase in heterotrophic microbial activity due to biodegradation of organic constituents of the soil or waste can affect the mobilization of radionuclides. Heterotrophic bacteria and fungi can release metals from various materials, including copper-nickel concentrates, low-grade copper ore, uranium from granites, and potassium from Lucite. Several mechanisms were proposed to explain the heterotrophic microbial solubilization of

insoluble metals, including the production of organic acids, the formation of chelates, and the metabolism of metal associated anions. Leaching of metals by heterotrophic organisms is due entirely to the chemical reactions of the excreted microbial metabolites and decomposition products. In many cases, a combined effect is important, for example, when organisms secrete organic acids that may have a dual effect in increasing metal dissolution by lowering the pH, and by complexation. A wide variety of heterotrophic microorganisms may be involved in solubilizing uranium from granitic rock where it generally occurs as an oxide (Bhurat et al., 1973). Such solubilization is caused by the production of organic-acid metabolites, such as oxalic-, isocitric-, citric-, succinic-, hydrobenzoic-, and coumaric-acids via their carboxylic- and phenolic-groups (Berthelin and Munier-Lamy, 1983; Bloomfield and Kelso, 1973; Bloomfield and Pruden, 1975; Bloomfield et al., 1971; and Bolter et al., 1975). A *Bacillus sp*. able to reduce iron was shown to anaerobically mediate the solubilization of hydrous PuO_2 in the presence of a complexing agent, nitrilotriacetic acid (Rusin et al., 1994).

When microorganisms are grown in an iron-deficient medium, they elaborate specific iron chelators, such as siderophores. Iron-sequestering agents could play an important role in the complexation of radionuclides, and so increase their solubility. For example, the dissolution of plutonium dioxide was enhanced in the presence of Desferol, a polyhydroxamate chelate produced by microorganisms. *Pseudomonas aeruginosa* CSU, isolated from a Pu-contaminated pond at Rocky Flats, elaborated several metabolic products that complexed uranium and thorium (Premuzic et al., 1985).

20.3.2 Immobilization of radionuclides and metals. Radionuclides and toxic metals are immobilized by (i) the bioaccumulation and biosorption by microbial biomass and biopolymers; (ii) mineral formation; and, (iii) precipitation due to the enzymatic oxidation

and reduction of metals. These processes received much attention because of their potential application in treating the secondary aqueous waste-streams containing radionuclides.

(i) Bioaccumulation and Biosorption. The ability of microorganisms and the polymers they secrete to scavenge metal ions has been studied intensively. Microbial bioaccumulation was reported for lead, silver, platinum, palladium, gold, mercury, gallium, cadmium, copper, nickel, as well as the radionuclides cobalt-60, cesium-137, strontium-85, uranium, thorium, radium, americium, and plutonium. Microorganisms, living or dead, possess an abundance of functional groups on their cell surface that bind metal ions. Intracellular accumulation of metals was observed among all classes of microorganisms and usually occurs by an energy-dependent transport system. Polymers secreted by many actively metabolizing microbes also immobilize metals. Indeed, metal-binding to cell surfaces and polymers is a promising technology for bioremediating contaminated surface and ground waters. The mechanisms of metal binding by microbial cell walls and extracellular substances and the applications of these materials in bioremediation of radionuclide and toxic metal waste streams was reviewed elsewhere (Beveridge and Doyal, 1989; Hughes and Poole, 1989; Volesky, 1990; Brierley, 1991; Francis 2004).

(ii) Precipitation of metals by sulfate-reducing bacteria. Sulfate-reducing bacteria oxidize organic matter and reduce sulfate to sulfide in anaerobic environments. The hydrogen sulfide (H_2S) so produced reacts with metals to form water-insoluble, metal- sulfide compounds. The precipitation of metals by sulfate-reducing bacteria in the natural environment and in treating waste water is well documented and is being exploited in engineered natural systems, such as constructed wetlands, to treat metal contaminants (Brierley, 1991).

(iii) Biocrystallization: Bacteria interact very strongly with metal ions and immobilize and concentrate them, eventually generating very small minerals. Microbial biofilms not only

bind significant quantities of metallic ions naturally, but they also serve as templates for the precipitation of insoluble mineral phases. In nature, bacteria are constantly involved in producing minerals in soils and sediments. A *Citrobacter sp*. accumulates heavy deposits of metal phosphate, derived from an enzymatically liberated phosphate ligand. The cells exhibit no constraints on saturations and can accumulate several times their own weight of precipitated metal. This high capacity is attributable to biomineralization. For example, uranyl phosphate accumulates as polycrystalline HUO_2PO_4, at the cell's surface. The precipitation of uranium and other toxic metals by *Citrobacter sp*. was demonstrated with enzymatically liberated inorganic phosphate (Macaskie, et al. 1992).

(iv) Immobilization due to reductive processes. The reduction of an element from a higher to a lower oxidation state or to elemental form affects its solubility, so resulting in the precipitation of several metals. For example, the following conversions by microorganisms have been reported: Selenate, selinite, tellurate, and tellurite to elemental forms; vanadate to a vanadyl compound; molybdate and molybdenum trioxide to a molybdenum-blue compound; arsenate to arsenite; mercuric chloride to elemental mercury; chromate ions to chromic ions that are precipitates at neutral pH; pentavalent- and trivalent-bismuth to an elemental form; lead dioxide to its divalent state; osmium tetroxide to the osmate ion; osmium dioxide and trivalent osmium to the metal; and hexavalent uranium to the tetravalent state; hexavalent Pu to the tetravalent form, petavalent neptunium to the tetravalent form, and heptavalent technetium to tetravalent form (Mohaghehgi et al., 1985, Lovely, 1993, 1994; Woolfolk and Whitely, 1962; Kauffman et al., 1986; Francis et al., 1991a,b, 1994, 2008; Neu et al., 2005 Francis et al., 2008; Deo et al 2011). Basic information on the mechanisms of microbial transformations of radionuclides is useful in understanding the migratory behavior of radionuclides from the waste repository sites, in predicting their environmental fate and transport, in the long-term management of the contaminated sites, in remediation of

contaminated sites, and in developing appropriate biotreatment technology, including the recovery and recycling of elements, the stabilization of wastes, and volume reduction, and minimization.

20.5 *Ex-situ and In-situ* remediation studies of contaminated soils

Radionuclide-contaminated sites can be remediated by using techniques designed to extract or segregate the contaminated fraction from the rest of the soil, either in-situ- or ex-situ. *Ex situ* bioremediation involves excavating the contaminated material and its treatment in above-ground facilities located on site or offsite, whereas *in situ* remediation is undertaken at the site of contamination. E*x situ* methods involves extraction separation, recovery of radionuclides, treatment of secondary waste-stream, and the proper disposal of the solid wastes. The *ex-situ* treatment processes are better understood; hence, they are relatively easy to implement, monitor, and control. The treatment of radionuclide contaminated soils, sediments, and wastes involve excavation followed by ex-situ treatment or disposal. The common *ex-situ* treatment for excavated soils is solidification/stabilization.

In-situ remediation technologies have several advantages over *ex situ* methods as they are cheaper, and less disruptive because no excavation is required. In addition, the exposure of site workers to hazardous contaminants is minimal. *In situ* bioremediation methods are suitable for mitigating widespread pollution that is, present in dilute concentrations, or otherwise inaccessible. However, *in-situ* treatment methods for radionuclide-contaminated soil have not been fully explored because of their radioactivity and the uncertainty in containing the radionuclides over the long-term.

Radionuclides can be extracted by exposing the contaminated soil to a solution containing extracting agents (washing the soil, and its *in-situ* flushing). Soil washing removes metals by chemical- or physical- treatment in aqueous suspension. Soil washing is an ex situ

process that necessitates requires soil excavation prior to treatment. Chemical treatment involves adding extraction agents that react with the contaminant and leach it from the soil. The contaminated fraction of soil and/or process water is separated from the remaining soil, and disposed of or treated. It includes soil decontamination as well as treating the impregnated extracts or the secondary waste by biological means.

Bioleaching uses microorganisms directly to solubilize metal contaminants, or as a result of interactions with the metabolic products, or both. Bioleaching can be used in-situ or ex-situ to aid in removing metals from soils. This process is being adapted from the mining industry for using in the remediation of metals.

To date, the majority of the technologies that have been investigated for remediating radionuclides are ex-situ technologies. Much of the contaminated soils are removed and stored, or depending on the radioactivity and its concentration, they are disposed of at a low- or intermediate-level waste facility. If the concentrations are low but the large volume of the waste makes it prohibitively expensive to dispose of as LLW then chemical and biological treatments are employed to selectively remove of the radionuclides from the soil and return it to normal use; thereafter, secondary treatment of the liquid waste to concentrate the extracted radionuclides and dispose them as appropriate is an attractive option.

The microbial transformations of selected radionuclides of interest in contaminated soils, sediments, and wastes relevant to in-situ and or ex-situ bioremediation technologies are summarized below.

20.5.1 Thorium biotransformation and remediation

Thorium is a natural radioactive element predominantly found in a single isotopic form, viz., ^{232}Th. It is an alpha emitter with a half-life of about 14.05 billion years. Thorium is nearly three times more abundant than uranium in the Earth's crust, and s present in minerals such

as thorite (ThSiO$_4$), thorianite (ThO$_2$+UO$_2$) and monazite. Thorium is extracted from monazite along with the rare-earth metals. Unlike uranium, information is lacking on the impact of thorium mining and processing. Mining and refining it may lead to the contamination of soil and water. Thorium is only stable at its +4 oxidation state. It readily undergoes hydrolysis in water and forms colloids and polynuclear-species.

Microbe – thorium interactions have received little attention, possibly because thorium is not used currently in commercial nuclear reactors. However, there is a growing interest worldwide in building thorium-based reactors. The role of microbial activities in the dissolution and immobilization of thorium is unknown.

The biosorption of thorium by by a Pseudomonas strain (Kazy et al. 2009), and by the fungi *Rhizopus arrhizus* and *Aspergillus niger* was about 116 - 180 mg/g biomass; furthermore, it was shown that Th^{4+} coordinates with the nitrogen of the fungal chitin cell (Tsezos and Volesky 1981, 1982; White and Gadd 1990). The biosorption of Th by dry biomass of *Sargassum filipendula* was 2.59 µg Th / g in a fixed-bed reactor (Picardo et al., 2008). Co-precipitation for removing Th from water was explored using exopolymeric substances produced by a soil bacterium *Bradyrhizobium* (*Chamaecytisus*) strain BGA-1 (Diaz-Marrero et al., 2004). Various species of bacteria, actinomycetes, and fungi were screened for the accumulation of thorium ion from water; the maximum accumulation was observed in the cells of *Micrococcus luteus* (Nakashima and Tsuruta 2004). Alginate polymers were effective in removing and recovering Th (IV) from solution; they alginate polymers removed 169 mg Th/g.
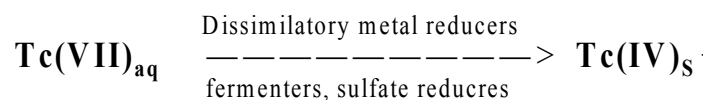
Several metabolic products capable of complexation with Th were elaborated when *Pseudomonas aeruginosa* was grown in medium containing Th (Premuzic et al., 1985). Recently, Desouky, et al (2011) reported that the metabolic bioproducts from *Aspergillus ficuum* and *P. aeruginosa* extracted thorium (Th^{4+}), uranium (UO$_2{}^{2+}$) and rare earth elements

(REEs) from a thorium–uranium concentrate. *P. aeruginosa* produced an element-specific ligand (siderophore) that changed the pH and enhanced the chelation of Th^{4+} and UO_2^{2+}. The siderophore generated at pH 5.3 can bioleach and complex with 68% of uranium and 65% of thorium. Also, *A. ficuum* produced different kinds of organic acids that leached 30% of uranium and 29% of thorium in addition to 20% of lanthanum, 33% of cerium, and 2.5% of yttrium as rare-earth elements at pH 3.0 (Desouky, et al 2011). Organic acid metabolites, such as citric acid, produced by *A. niger* form stable complexes with Th (Bobtelsky and Graus, 1954). These studies clearly showed that microbial activities can affect the solubility of Th and demonstrated their potential use in remediating Th-contaminated soils.

20.5.2   *Technetium biotransformation and remediation studies*

Technetium - 99 is a β-emitting radionuclide with a half-life of 2.1×10^5 years. It is released into the environment from nuclear power reactors, nuclear wastes, reprocessing facilities, and from testing nuclear weapons. It exits in as many as 19 isotopes, and in multiple oxidations states (0, +3, +4, +5, +6, +7). Of these, the stable heptavalent pertechnetate ion (TcO_4^-) and the quadrivalent Tc(IV) ion are of environmental concern. Pertechnate (TcO_4^-) ion is the most stable and soluble ion in aqueous environments.

Technetium can undergo oxidation–reduction reactions under environmental conditions. It can be reduced chemically and biologically. The reduction and precipitation of pertechentate anion is carried out anaerobically by a wide variety of microorganisms (Lolyd and Mcaskie 1996; Henroit 1989; Pignolet et al., 1989; Francis et al., 2002) and that bacteria are able to enzymatically reduce soluble Tc(VII) to insoluble Tc(IV) under anaerobic conditions (Lolyd and Mcaskie 1996). A *Halomonas* strain isolated from a seawater removed Tc(VII) by aerobically reducing it to Tc(IV) (Fujimoto and Morita, 2006).

$$Tc(VII)_{aq} \quad \xrightarrow[\text{fermenters, sulfate reducres}]{\text{Dissimilatory metal reducers}} \quad Tc(IV)_S$$

The predominant chemical species identified include TcO_2, $TcO(OH)_2$, and TcS_2 depending on the type of microorganism involved. In addition, microorganisms can mediate the reduction of Tc(VII) to Tc(IV) indirectly by generating ferrous iron and sulfide. For example, the sulfate reducers reduce pertechnetate to insoluble TcS_2. Other organisms are known to generate Tc oxide (TcO_2) and oxyhydroxide species [$TcO(OH)_2$] anaerobically. In culture medium, Tc is associated with the cells and with macromolecules as reduced Tc-organic complexes. Pignolet et al. (1989) observed that Tc was bound to high-molecular-weight cellular constituents in pure- and mixed-cultures of bacteria isolated from marine sediments. Henrot (1989) reported the association of Tc with bacterial polysaccharides in anaerobically grown bacteria, and stated that the reduction of pertechnetate was metabolically linked, and not due to changes in the redox conditions. About 70% of the total Tc was associated with the bacteria and/or precipitated. The remaining Tc in soluble form was associated with organics. The anaerobic reduction of soluble Tc(VII) to Tc(IV) by *Clostridium* sp precipitated Tc. The reduced Tc was associated with the cell biomass, as well as being in solution associated with organic metabolic products. Also, the reduced Tc(IV) formed a soluble Tc-DTPA complex when DTPA was added to the medium of *Clostridium* species (Francis 2002).

$$Tc(VII)_{aq} \quad \xrightarrow{\textit{Clostridium} \text{ sp.}} \quad \textbf{Tc(VII)-organic complex}$$
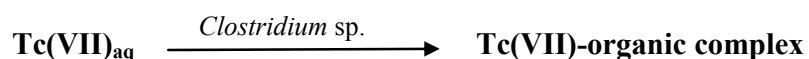
Technetium adsorption by soils and sediments has been attributed to microbial activity. Landa et al. (1977) reported eight out of eleven soils tested adsorbed 98% of the

pertechnetate within 2 to 5 weeks, and that sterilizing the soil eliminated this adsorption. Tagami and Uchida (1966) investigated the influence of microbial activity on plant- available Tc under aerobic- and anaerobic-(waterlogged) conditions in soil amended with 0, 0.05% and 0.5% glucose and compared them with sterile soil. They found that Tc was bound to the soil as a result of changes in the redox caused by microbial action. Sheppard et al. (1990) noted the negligible sorption of TcO^-_4 in aerobic soils, and substantial sorption of reduced Tc in anaerobic environments, especially in the presence of organic matter; they suggested that reduced Tc may be transported as a complex with organic ligands. Peretrukhin et al. (1996) showed that Tc was sorbed on to the sediments of a lake in Russia by the action of sulfate-reducing bacteria. Biogenic hydrogen sulfide converted the readily soluble sodium pertechnetate to poorly soluble Tc(VII)- and Tc(IV)- sulfides.

The Tc(IV) forms complexes with naturally organic compounds, such as acetate, oxalate, citrate, and humic acids, and with the synthetic chelating agents EDTA, and DTPA that can increase their solubility. The nature and stability of the Tc-organic complexes is not understood. Moreover, the ability of the bacterium to metabolize Tc-organic complexes, such as Tc-DTPA and Tc-citrate, is not known. These studies suggest that Tc may be present as insoluble or soluble form or as colloids, depending on the type and extent of bacterial activity in subsurface environments; hence, the potential exists for the transport of reduced Tc in these forms.

However, the reduced Tc(IV) in soils and sediments can be remobilized by re-oxidation to Tc(VII) depending on the nature of the oxidant present in the systems (Gu et al., 2011).

20.5.3   *Radium biotransformation and remediation studies*

Radium is naturally occurring radionuclide formed by the decay of uranium in the environment. In nature, radium occurs in trace quantities in uranium ores. All isotopes of

radium are highly radioactive, but Ra^{226} is the most stable isotope. It has a half-life of 1601 years, and is an alpha emitter with accompanying gamma radiation. Microorganisms indirectly can affect the environmental mobility of radium.

At many uranium mining and milling sites, soluble radium is released and must be removed from the effluents prior to their discharge. Co-precipitation, by adding barium chloride $(BaCl_2)$ to sulfate-rich tailing effluents, is the method employed for removing radium as $(Ba,Ra)SO_4$. The precipitate is allowed to settle, resulting a radioactive sludge, and supernatant free of Ra^{2+} ready for discharge to the environment (McCready et al., 1980).

20.5.3.1 *Radium coprecipitation with barium sulfate.* At many uranium mining and milling sites, soluble radium is removed as a co-precipitate with $BaSO_4$ by adding $BaCl_2$ to sulfate-rich tailing effluents. The resulting $(Ba,Ra)SO_4$ precipitate is allowed to settle, yielding a radioactive sludge and supernatant that is sufficiently low in $^{226}Ra^{2+}$ to be discharged. The disposal of radioactive sludges must ensure that $^{226}Ra^{2+}$ does not leach into the groundwater because microbes might transform the stabilized radioactive wastes into mobile compounds. For example, Ra, co-precipitated with $BaSO_4$, was solubilized anaerobically by sulfate-reducing bacteria (Fedorak et al., 1986).

$$(Ba,Ra)SO_4 \xrightarrow{\textit{sulfate – reducing bacteria}} {}^{226}Ra^{2+} + Ba^{2+} + H_2S$$
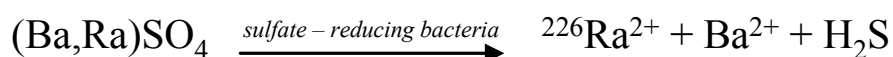
$BaSO_4$ was solubilized under anaerobic conditions by bacteria (Bolze et al., 1974). High Ba^{2+} concentrations in aquifers have been attributed to sulfate reduction by *Desulfovibrio vulgaris*. Thus, McCready and Krouse (1980) showed that *D. vulgaris* could release H_2S, $^{226}Ra^{2+}$, and Ba^{2+} from a $(Ba,Ra)SO_4$ sludge. Studies of such sludges from two Canadian uranium mine and mill sites revealed that after adding usable carbon, SO_4^{2-} was reduced to S^{2-}, with a concurrent release of $^{226}Ra^{2+}$, Ba^{2+}, and Ca^{2+} due to enhanced anaerobic microbial activity. Levels of dissolved $^{226}Ra^{2+}$ reached approximately 400 Bq/L after ten

weeks of incubation. Thus, for ultimately disposing of these sludges, conditions must be maintained that minimize the activity of the indigenous sulfate-reducing bacteria to ensure that $^{226}Ra^{2+}$ is not released.

Aerobic treatment of the effluents from uranium mills containing toxic amounts of Mn^{2+} and ^{226}Ra with *Arthrobacter sp*., precipitated $\sim 92\%$ of the Mn^{2+} as the hydrous oxide of manganese; along with it, about 95% of the ^{226}Ra was co-precipitated (Mathur and Dwivdey, 1988). However, under anaerobic conditions, Mn and ^{226}Ra can be remobilized by the bacterial reduction of manganese oxide (Mn(IV) to Mn(II); this phenomenon must be taken into consideration for waste disposal, particularly under anaerobic conditions, such as in landfills and subsurface disposal-facilities where conditions become anoxic.

These studies show that the disposal of wastes containing radionuclides and metals co-precipitated with iron- and manganese-oxides, may entail the extensive leaching of iron, toxic metals, and radionuclides due to enhanced anaerobic microbial activity.

20.5.4   *Polonium biotransformation and remediation*

Polonium-210 is a very rare highly radioactive, toxic element that occurs naturally in association with U and Th minerals. It has a short half-life of 138 days, and is chemically similar to bismuth and tellurium. It is found in uranium ores at about 0.1 mg per metric ton (1 part in 10^{10}), i.e., approximately 0.2% of the abundance of radium. Different isotopes of Po are produced in the decay of ^{238}U, ^{235}U, and ^{232}Th. It is the last radioactive member of the ^{238}U decay series, and can be measured in very low quantities using alpha spectroscopy. ^{210}Po can be an environmental pollutant arising by release from uranium-containing phosphorite – phosphate and phosphogypsum – phosphoric acid production. Polonium exists as +2 and +4 oxidation states. Sulfate-reducing bacteria mobilize the Po contained in phosphogypsum due to reduction of the sulfate in the gypsum under anaerobic conditions. Organic ligands

produced by aerobic bacteria are also known to mobilize Po by forming Po-organic complexes.

Immobilization of Po by sulfate-reducing bacteria is due to H_2S production and the precipitation of Po as polonuim sulfide (PoS). Bacterial uptake of Po extracellularly or intraellulary has been reported (Larock et al 1996). Such immobilization in nature must be considered as transient if, upon the death of these cells, the Po becomes re-dissolved in the bulk phase.

Because of the chemical similarity of Po to S, studies were begun to determine whether bacteria, particularly those species active in sulfur cycling, could account for the selective solubilization and mobilization of Po. Possible sources of Po are the U-rich phosphate rock and phosphogypsum (gypsum), a byproduct in manufacturing phosphoric acid. Experiments involving the interaction of bacteria with this waste gypsum demonstrated the solubilization of Po. Sulfate-reducing bacteria particularly were effective at mediating Po release, provided that the sulfide levels did not rise above 10 μM, in which case Po apparently was co-precipitated as a metal sulfide. Conversely, the ability of sulfate-reducing bacteria to effectively remove dissolved Po when sulfide levels are high suggests that these bacteria may serve as an effective bioremediation tool in reducing Po levels in groundwater.

The biomethylation of Po by microbes to dimethyl polonide (DMPo) in the presence of methylcobalamine was observed. The loss of Po was noted from cultures of bread mold and non-sterile groundwater in Florida. Volatile Po has been trapped from the headspace of cultures inoculated with sediments and pure cultures of bacteria, and the volatile Po compound has been identified as dimethyl polonide (Bahrou et al., 2012).

20.5.5   *Cesium biotransformation and remediation studies*

Cesium is a metal that occurs naturally in various minerals in a nonradioactive stable form, or as produced from uranium and plutonium during fission in nuclear reactors that is radioactive and unstable. The commonest radioactive forms of cesium are ^{137}Cs and ^{134}Cs. Cesium -137 contamination of the environment is much more significant than that of ^{134}Cs; it mainly is contained in the topsoil layer. Cesium-137 was introduced into the environment from the fallout from atmospheric tests of nuclear weapons in the 1950s and 1960s, (much of it now has decayed), nuclear-reactor waste, spent nuclear-fuel wastes from reprocessing plants, and accidental releases, such as the Chernobyl accident in the Ukraine and in Fukushima, Japan. Cesium-137 undergoes radioactive decay, with the emission of beta particles and relatively strong gamma- radiation, to barium-137m, a short-lived decay product, which, in turn, decays to a nonradioactive form of barium. The half-life of ^{137}Cs is 30.17 years.

Cesium is a structural analog of K. Much of the Cs deposited on the litter layer leach into mineral soil and remains associated with the soil's mineral phases, and expectedly, perhaps only small fraction will be bioavailable. ^{137}Cs resides predominantly on "strong" sites on clay interlayers. Cleaning up of contaminated soils generates a large volume of radioactive waste. In some instances, large contaminated areas might be mitigated by burying and covering the contaminated substances with concrete, soil, or rock to prevent the contamination from spreading further into the environment. Stripping out ^{137}Cs from contaminated soil efficiently and without destroying the soil organic content dramatically could lower the volume of waste and the cost of disposal.

Cesium bound to interlayer sites is not readily exchanged by other cations, and generally is considered fixed (Comans et al., 1991). A strong surface association between Cs and the soil's solid phase occurs at frayed edge sites (FES) of micaceous phyllosilicate minerals. Plant root and microbial exudates may enhance Cs bioavailability in the rhizosphere by accelerating weathering at the frayed edges of phyllosilicate minerals, and

releasing Cs sorbed to frayed edge- and interlayer-sites, so making the ion available for uptake. Many studies documented the significance of exudates from plant roots and rhizosphere microorganisms to phyllosilicate mineral-weathering processes in the soil environment (Wendling et al., 2004; Staunton and Levacic, 1999; Kim et al., 1997; Robert, M.; Berthelin, 1986; Berthelin, 1983). Oxalate affected Cs interactions with illite (Wendling et al., 2004).

Biogeochemical processes in the rhizosphere can greatly alter the interactions between contaminants and soil minerals. Cesium desorption from illite in the presence of exudates from bacteria found in the rhizosphere of crested wheatgrass (*Agropyron desertorum*) reveal the potential contribution of bacteria to illite weathering in the rhizosphere of crested wheatgrass. Exudates from three strains of bacteria belonging to the genera Bacillus, Ralstonia, and Enterobacter significantly enhanced Cs desorption from illite. In addition, Cs desorption increased with rising concentrations of the Bacillus exudate. Cesium desorption from illite as a function of both exudate type and concentration was positively correlated with Al dissolution, suggesting that the Al-complexing ability of the exudates played an important role in enhancing Cs desorption. The density of frayed edge sites (FESs) on illite increased after treatment with bacterial exudates, while the Cs/K selectivity of FES decreased. These results suggest that exudates from bacteria isolated from the rhizosphere can enhance Cs desorption from frayed edges of illite and, therefore, can alter Cs availability in micaceous soils. Soil decontamination using microbes is an option that is being considered. Cs-137 mimics that of sodium and potassium, which are readily absorbed by the cells. Cesium-accumulating bacteria isolated from soil display the rod-coccus growth cycle and contain mesodiaminopimelic acid, mycolic acids, and tuberculostearic acids (Tomioka, et al. 1992). Because monovalent cation uptake is usually energy dependent, cesium accumulation by bacterial strains may be energy dependent. Therefore, the mechanism of cesium removal by

these strains is not simple adsorption. The optimum pH for Cs uptake by the strains *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. Strain CS402 was 8.5. Rubidium and Cesium assume part of the role of potassium in the growth of both strains. Potassium and rubidium inhibited Cs accumulation by these strains. It is likely that both strains accumulated Cs through potassium transport system (Tomioka, et. al. 1994). Recently, adsorption of cesium by unicellular green algae was reported (Shimura et al., 2012). Nevertheless, the nature of Cs association with the cells (extra or intra cellular) remains unclear, as does the long-term fate of bio-associated Cs. Very high radiocesium activities have been observed in the fruiting bodies of several fungal species since the Chernobyl accident (Dighton and Horrill 1998).

Sasaki et al (2013) demonstrated that the terrestrial cyanobacterium Nostoc (N) commune can absorb high levels of radioactive cesium. This species is a heterocystous blue-green algae that forms jelly-like clumps of polysaccharides. Radioactive material is presumed to be deposited in them simplifying its removal from the environment. Furthermore, this species reportedly grows under high radiation-exposure. Sasaki et al (2013) monitored the accumulation of radioactive material in the N. commune. In Nihonmatsu City, Fukushima Prefecture, N. commune accumulated 415,000 Bq/kg dry weight 134Cs and 607,000 Bq kg-1 dry weight ^{137}Cs. The concentration of cesium in the N. commune tended to be high in areas where the soil's radioactivity was high. A cultivation experiment confirmed that N. commune absorbed radioactive cesium from polluted soil. These data demonstrated that radiological absorption using N. commune might be suitable for decontaminating polluted soil. Because this species is a heterocystous blue-green algae that forms jelly-like clumps, it can be easily collected from the soil's surface. Furthermore, its weight decreases by about 90% when it is dried. Radiological absorption by N. commune may be a viable strategy for decontaminating polluted soil.

Radio-cesium is known to be bioavailable in forest ecosystems for long time, and it is necessary to intervene with the cycling process to decontaminate the forest ecosystem. Ecological processing to recycle radioactive Cs in a forest ecosystem is a viable approach to enhancing the decontamination of radio-Cs. Mushrooms accumulate high concentrations, and although the mushroom biomass in a forest ecosystem is small, the fungal mycelium in detritus and soil is large, and thus fungi contain substantial amounts of radio-Cs. It is well known that concentration of some nutrients, such as nitrogen and phosphorus increase, whereas potassium decreases during the decomposition of leaf litter. Nobuhiro et al., (2013) recorded the concentrations of radio-Cs during the decomposition of leaf litter on a forest floor where ^{134}Cs and ^{137}Cs, respectively, in the surface soil was 5,700-, and 6,800-Bq/kg. They placed 16 g (dry wt) of the newly fallen mixed deciduous leaf litter (half of which was oak, *Quercus serrata*) from a deciduous forest about 50 km from Fukushima NPP into 25 cm x 25 cm litter bag. The ^{137}Cs concentration in the fresh litter was ca. 1,000 Bq/kg in December 2011. During the process of decomposition on the forest floor, the Cs in the litter increased exponentially and exceeded 10,000 Bq/kg after 6 months, indicating that Cs and K show contrasting dynamics during the early decomposition phase. An increase in fungal biomass in the early stage of litter decomposition also was observed. Therefore, this upward movement of Cs from the humus and soil layer points to the fungal translocation of nutrients from outside the litter's substrate. Retrieving the litter after 6 months showed a loss of removed 18% of ^{134}Cs.

In the long term, secondary contamination by the wash off of ^{137}Cs from contaminated soils, and its remobilization from sediments continues at a much lower level. Soils with high organic content may release much more radiocaesium to surface waters than do those of the mineral soils. Although the physical processes (radionuclide removal, mixing with soil) and chemical processes (soil liming, fertilization) involved in environmental remediation

technologies are well understood, many of the biological processes that could be used in remediating contaminated soils are not fully understood.

20.5.6 Strontium biotransformation and remediation studies

Strontium-90 is a high-yield fission product. It is a β emitter with a half-life of 29.1 years. The biochemical behavior of strontium is similar to that of calcium, and therefore, it tends to concentrate in the bones and teeth. Strontium-90 is an important radionuclide contaminant at nuclear facilities and nuclear-accident sites. In the natural environment, strontium exists solely as Sr^{2+}. The geochemical behavior of strontium is similar to that of Ca^{2+} and Sr speciation is not influenced directly by changes in redox conditions. However, processes such as biosorption, bioaccumulation, and bio-precipitation have been investigated for developing bioremediation strategies for removing or immobilization of Sr from contaminated water or sites.

In soils, ^{90}Sr is bound as an exchange complex to clay minerals, or exists as a fixed component of the soil's organic matter, iron (hydr)oxides, or insoluble carbonate or phosphate. Microorganisms can affect the association of various forms of Sr in soils by (i) dissolution of the carbonate- and phosphate-phases, clays, and other minerals due to the production of organic acids and sequestering agents; (ii) reductive dissolution of iron and the release of Sr associated with the iron oxides, (iii) biodegradation of the organic carbon associated the Sr fractions; and, (iv) immobilization due to precipitation reactions i.e., the formation of strontium carbonate (Andersona nd Appana 1994), microbial formation of strontium calcite phase at a groundwater discharge zone (Ferris et al 1995), and by biomass/exopolymers.

Bioaccumulation of Sr as structural analogue for Ca was reported for several microorganisms (Page and Doran, 1981; Strandberg et.al., 1981; Faison, et al. 1990).

Consequently, there is considerable interest in using microorganisms to remove radioactive Sr from waste streams. Sr-binding activity in *Microccous luteus* is localized on the cell envelope, and is sensitive to pretreatment. Bound Sr can be displaced by chelating agents, divalent cations, or H^+ (other monovalent cations are less effective at displacing Sr). Strontium binding in *M. luteus* is reversible, though both ion exchange, mediated by acidic cell surface components, and intracellular uptake may be involved (Faison, et al. 1990). Biosorption of strontium was reported by bacterial- and algal-cultures (Shimura et al., 2012). In addition, accumulation of strontium into barite in the form of $(Ba,Sr)SO_4$ was shown via the for selective removal of strontium using unicellular freshwater green algae (Krejci et al., 2011). Strontium was accumulated as $SrSO_4$ crystals in the vacuole of the desmids.

Microbially induced calcium carbonate precipitation (MICP) was offered as a promising approach for the cleanup of radionuclide-contaminated sites, particularly for Sr bioremediation (Fujita, et al., 2010; Thorpe, et al., 2012; Archal et al., 2012). MICP is driven by microbial metabolism, and can be implemented using four different mechanisms, that are bacterial ureolysis, sulfate reduction, fermentation of fatty acids, and denitrification. Among them, only denitrification does not produce toxic byproducts. Incorporating strontium into calcite ($CaCO_3$) in artificial groundwater was studied using urease-producing bacteria (Warren et al., 2001; Fujita et al., 2004). MICP via biological denitrification is a more environmentally friendly and promising approach because it does not add additional pollutants such NH_4, as in the case of a urease- based approach. It can easily be performed under anoxic conditions, and has greater carbonate yield per mole of electron donor, facilitating the formation of $SrCO_3$ precipitates. Nitrate often is present as a co-contaminant with radionuclides in contaminated environments. Hence, Thorpe et al., (2012) investigated Sr^{2+} sorption and precipitation during denitrification in sediment microcosm experiments, and found that it caused the pH to increase (>9), resulting in decreased Sr^{2+} solubility.

20.5.6.1 *Microbiologically induced calcite precipitation (MICP)*. The overall reactions involvied in bioremediation process include the urease-producing NH_4^+ and HCO_3^-, desorption of Ca^{2+} and/or Sr^{2+} from solid surfaces by the NH_4^+ - and HCO_3^- promoted precipitation of $CaCO_3$ and co-precipitation of ^{90}Sr (Wu et al., 2011). Indigenous or introduced microorganisms induce the *in-situ* $CaCO_3$ precipitation. The following chemical reactions are proposed.

20.5.6.1.1 *Urease-driven strontium precipitation*: Microbially catalyzed hydrolysis of urea produces ammonia, bicarbonate, and increases the pH.

$$NH_2(CO)NH_2 + 3H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + HCO_3^- + OH^-$$
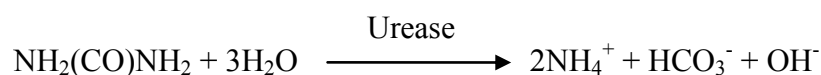
Bicarbonate promotes the precipitation of $CaCO_3$ in the presence of dissolved calcium with the formation of calcite.

$$Ca^{2+} + 2HCO_3^- \rightleftharpoons CaCO_3 + H_2O + CO_2$$
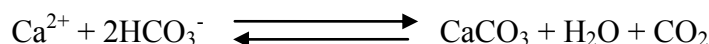
The precipitation of $CaCO_3$ is influenced by the concentration of Ca, dissolved inorganic carbon, pH and the presence of nucleation sites. Bacterial cells serve as nucleation sites and precipitation $CaCO_3$ on bacterial cell surfaces has been reported (Fujita et al., 2000; Ferris et al., 1987).

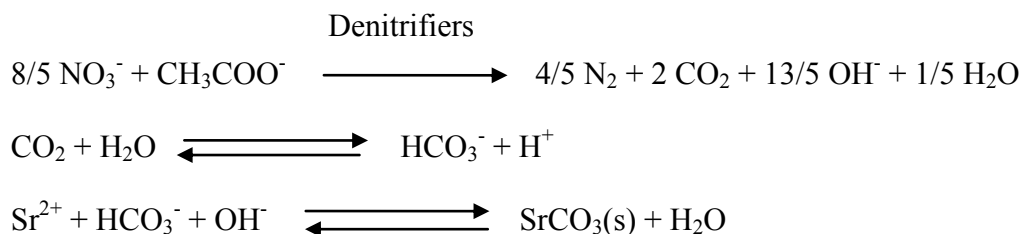Ammonium ions promote the exchange of sorbed Sr.

$$\text{Solid - } Sr^{2+} + 2NH_4^+ \rightleftharpoons \text{Solid} - (NH_4^+) + Sr^{2+}$$
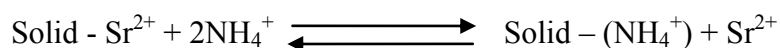
Sr co-precipitates into calcite and thus is removed from the aqueous medium.

$$x Sr^{2+} + (1-x)\, Ca^{2+} + 2HCO_3^- \rightleftharpoons Ca_{(1-x)}Sr_xCO_3 + H_2O + CO_2$$
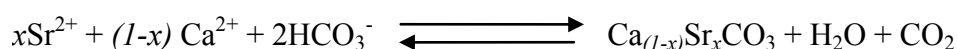
Fujita et al., (2000, 2004) used ureolytic bacteria that precipitate calcium carbonate to immobilize strontium. Strontium was integrated into the calcite structure through substituting for calcium by forming strontium-carbonate minerals that have very low solubility (Fujita et al., 2000, 2004). The effects of adding molasses and urea were tested for stimulating microbial hydrolysis of urea in the Eastern Snake River Plain Aquifer, Idaho (Fujita et al., 2008). Diluted molasses was injected three times over two weeks to promote microbial growth. Thereafter, one injection of urea in a single-well experiment caused the total number of cells to increase by 1 to 2 orders-of-magnitude. Quantitative PCR assay revealed that numbers of urease genes (ureC) rose by 170 times above pre-injection levels. The researchers recovered calcite precipitates after injecting the urea. Overall, the data indicated the viability of manipulating biogeochemical processes to promote the immobilization of metals or radionuclides. The environmental conditions conducive to carbonate precipitation are not unique to any specific microorganism.

20.5.6.1.1   Denitrification-driven   strontium   precipitation. Denitrification involves the reduction of NO_3^- to N_2 and is driven by the presence of an electron donor (i.e. acetate). The microbial denitrification produces the alkalinity and carbon dioxide (CO_2) required for the MICP. Denitrification of nitrate produces almost 2 moles of CO_3^{2-} per mole of acetate (1.6 moles of nitrate).

$$8/5\ NO_3^- + CH_3COO^- \xrightarrow{\text{Denitrifiers}} 4/5\ N_2 + 2\ CO_2 + 13/5\ OH^- + 1/5\ H_2O$$

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

$$Sr^{2+} + HCO_3^- + OH^- \rightleftharpoons SrCO_3(s) + H_2O$$

Denitrifying bacteria are ubiquitous in the environment. The anoxic conditions of aquifer sediments of subsurface environments are ideal as bacteria use NO_3^- as an electron acceptor instead of O_2. Therefore, denitrification-driven MICP can be an effective bioremediation

approach for immobilizing radionuclides in situ or ex situ. This can be achieved by adding an exogenous electron donor (i.e., acetate) if nitrate is a co-contaminant.

Immobilization of Sr as $SrCO_3$ by bacteria was reported (Anderson and Appanna 1994). *Pseudomonas fluorescens* grown in a medium containing Sr-citrate metabolized citrate and precipitated Sr as crystalline $SrCO_3$ due to the production of CO_2 from citrate metabolism. This study demonstrated the potential of Sr for microbial immobilization in contaminated environments.

[90]Sr contamination is a major problem at several sites; at some, ^{90}Sr has migrated deep underground so complicating site remediation. A method based on mixing a solution of calcium citrate and sodium phosphate in soil was developed to immobilize ^{90}Sr in contaminated soils (Moore et al 2004). As the indigenous soil microorganisms mineralize the citrate, calcium is released and forms hydroxyapatite. Treating soils with a sodium phosphate/calcium citrate solution results in the formation of hydroxyapatite with CO_3^{2-} substitutions, with a formula of $(Ca_{4.8}Na_{0.2})[(PO_4)_{2.8}(CO_3)_{0.2}](OH)$. The average Sr uptake was 94.7% for soil treated with apatite; in desorption experiments, the apatite-treated soil released an average of 4.8% of it. These results show the potential of forming apatite in soil via soluble reagents for retarding radionuclide migration: Injecting a Ca-PO_4-citrate solution (with a Ca-citrate solution complex); the in situ biodegradation of citrate resulting in apatite $[Ca_6(PO_4)10(OH)_2]$ precipitation and coprecipitation of ^{90}Sr in pore fluid and solids in the treatment zone; adsorption of ^{90}Sr by the apatite surface; apatite recrystallization with ^{90}Sr substitution for Ca (permanent); and, the radioactive decay of ^{90}Sr to ^{90}Y to [90]Zr.

Studies also demonstrated that citrate biodegradation/apatite precipitation occurs in several sediments, resulting in the sequestration of U, Tc, Sr, and Pb. The solids are initially amorphous; however, laboratory tests show that they age to form crystalline apatite in a period of several weeks.

20.5.7 Iodine biotransformation and remediation

Radioactive iodine (^{129}I) is a long-lived fission product produced in significant amounts in nuclear power reactors. It has a half-life of 1.57×10^7 years and will be present in radioactive wastes and upon their storage in geological disposal facilities. Radioactive iodine is released into the environment from geological repositories, spent nuclear-fuel reprocessing plants, and in accidents at nuclear power plants.

The predominant chemical forms of iodine (I_2) in the environment are iodide (I^-) and iodate (IO_3^-), both of which are highly soluble and mobile in the environment. Iodate is the most stable form of iodine in seawater. Iodate can be reduced to iodide in the surface waters of seas, and this reduction appears to be linked to biological activity. Major mechanisms that govern the iodine cycle are the atmospheric volatilization of organic iodine compounds, the accumulation of iodine in living organisms, the oxidation and reduction of inorganic iodine species, and the sorption of iodine by soil and sediments. Microorganisms are known to influence the chemical behavior of iodine through various processes, such as volatilization in the form of organic – iodine compounds (i.e. CH_3I), the oxidation of iodide (I^-) to iodine (I_2), the reduction of iodate (IO_3^-) to iodide (I^-), and via bioaccumulation (Amachi 2008).

Incubating soil samples with varying levels of oxygen, water, and biomass levels demonstrated iodine immobilization. Pure cultures of bacteria and fungi isolated from the soil incorporate radioiodine (Bors and Martens 1992). Sorption of radioiodine was higher to soils that contained organic substances or biomass compared to clay minerals; the sorption process was irreversible (Bors and Martens 1992). Thus, growth and metabolic activities of microorganisms could have important effects on mobility of iodine in the natural environment.

In terrestrial environments, iodine concentrations accumulated in soils at ~5 mg kg–1 worldwide, which is much higher than those of their parent materials, such as rocks and

plants (0.05 to 0.5 mg kg–1), (Amachi et al. 2005). Such high iodine accumulation in soils and sediments was attributed, at least in part, to microbial effects, although the mechanism of the accumulation process is not fully understood. Here, one possible explanation is that the iodide ion (I⁻) is transported actively into the bacteria isolated from the marine sediment, which accumulate iodide >5000-fold (Amachi et al. 2005). Iodide adsorption by the Gram-positive soil bacterium *Bacillus subtilis* showed that positively charged single sites on the cell wall were responsible for iodide sorption on to the bacterial surface with a concentration of 3.54 ± 3.80 mmol iodide per gram of bacteria. The uptake and accumulation of iodide in washed cell-suspensions of marine bacteria increased when glucose was added, while iodate was not accumulated by the bacteria (Amachi et al. 2005). The adsorption of iodine by unicellular green algae was reported (Shimura et al., 2012). Although a wide variety of terrestrial- and marine-bacteria have the potential for fixing iodine in the environment, there is very little information on the mechanisms of microbial transformations of iodine, and on the chemical speciation and stability of the bioaccumulated iodine in bacteria.

20.5.8   Uranium biotransformation and remediation studies

Uranium occurs naturally in the earth's crust and is present in ores as uraninite and pitchblende as ^{238}U (99.27%), U^{235} (0.72%) and U^{234} (0.005%). It also occurs in secondary mineral phases associated with silicates, phosphates, carbonates, and vanadates. Uranium exists in four oxidations states (VI, V, IV, III), of which U(VI) and U(IV) are the predominant forms in the environment. Widespread uranium contamination of soil and water has resulted from front- and back-end of nuclear-fuel cycle, and from weapons production, nuclear research, coal combustion, mining, and application of phosphate fertilizers. In oxic environments, uranium primarily exists as soluble uranyl (U(VI)) species. In anaerobic environments, U(VI) is reduced to the less soluble immobile form U(IV). Microorganisms

play a significant role in the biogeochemical cycling of uranium by oxidation-reduction reactions, and thus regulate the environmental solubility and mobility.

20.5.8.1 *Uranium dissolution.* Autotrophic- and heterotrophic-microorganisms are known to solubilize uranium and their potential has been investigated for extracting and recovering uranium from ores. The iron- and sulfur-oxidizing bacteria catalyze the dissolution of uranium from ores and mill tailings. *Thiobacillus ferrooxidans* solubilizes uranium from ore by indirect action due to its generation of Fe^{2+}/Fe^{3+} and sulfuric acid (Munoz et al., 1993). Ferric iron and manganese oxide also can facilitate the oxidative dissolution of precipitated U(IV) to U(VI). Heterotrophic microorganisms indirectly solubilize uranium by producing CO_2, sequestering agents such as siderophores and organic acid metabolites, as well as by lowering the pH of the medium (Francis 1990).

20.5.8.2 *Uranium immobilization.* Soluble U(VI) is immobilized by facultative- and strict-anaerobes. They include dissimilatory metal reducers, sulfate reducers, and fermenters. The reduction of uranium was reported in axenic cultures of iron-reducing bacteria, fermentative bacteria, sulfate-reducing bacteria, cell-free extracts of *Micrococcus lactilyticus*, and in wastes by *Clostridium sp.*

$$U(VI)_{aq} \xrightarrow[\text{fermenters, sulfate reducers}]{\text{Dissimilatory metal reducers}} U(IV)_S$$
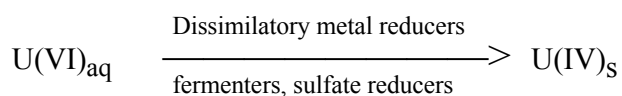
Studies showed that the biological reduction of U(VI) also could result in the formation of mononuclear U(IV) phases associated with C/N/O-, P-, or S-containing ligands, but the exact nature of these mineral phases is unidentified.

Determining the nature of the chemical species, such as the oxidation states, the organic- and inorganic-complexes, and the mineralogical association of uranium in

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November, 20, 2014

contaminated soils is important in assessing its long-term environmental mobility and stability. Several conventional techniques were used to determine the speciation of uranium; for example, hydrogen consumption, direct measurement of U(VI) in solution, conversion of insoluble species such as U(IV) to soluble U(VI) by acidification and oxidation, extraction from the aqueous phase using solvents, such as thenoyltrifluoroacetone, and the separation of U(VI) and U(IV) by ion-exchange resin. These methods are not reliable generally because they involve extensive sample preparation that can affect its oxidation state. X-ray spectroscopic (XRS) techniques require very little manipulation of the sample and provide more accurate information on the oxidation state of elements than do conventional methods. The speciation of uranium in microbial cultures has been followed by x-ray absorption near edge spectroscopy (XANES) and x-ray photoelectron spectroscopy (XPS) to show the reductive precipitation of soluble U(VI) to insoluble U(IV) by the anaerobic bacteria. Uranium was reduced only in the presence of growing or resting cells. Organic-acid metabolites, the extracellular components of the culture medium, and heat-killed cells failed to anaerobically reduce uranium (Francis et al., 1994). The change in free energy for reducing manganese and iron was -83.4 and -27.2kcal/mol CH_2O, respectively, while for reducing of hexavalent uranium to the tetravalent state, the change was -63.3kcal/mol, and to the trivalent state, it was 52.2kcal/mol. Uranium reduction should occur in the sequence Mn(IV) > U(VI) > Fe(III).

Contamination of soil and groundwater by uranium has been observed at or near sites where it is mined or processed. Several bacteria are known to reduce U(VI), but the our understanding of the biochemistry of this process is incomplete (Wall et al., 2006). The direct implication of microorganisms in the reduction of uranium is of considerable interest because of its potential application in bioremediating contaminated sites, in pretreating radioactive wastes, and in processes critical to the performance of nuclear-waste repositories. *In-situ* and

ex-situ bioremediation strategies are based on the microbial reduction of soluble U(VI) to insoluble U(IV). Numerous studies demonstrated that such reduction of uranium (VI) to uranium (IV) in groundwater and aquifer sediments is a viable *in-situ* bioremediation option to immobilize uranium. These studies highlighted the fact that indigenous bacteria can reduce uranium present in the natural environment but that microbial activities are limited by the availability of suitable electron donors. Therefore, to stimulate the *in-situ* microbial reduction of U(VI) to U(IV), electron donors such as acetate, ethanol, or lactate are injected at the contaminated sites.

20.6.   *In-situ* and *ex-situ* immobilization of uranium by anaerobic bacteria

Immobilization or stabilization of radionuclides is accomplished by chemically or biologically converting them to insoluble and environmentally stable forms. Microbial reduction of soluble hexavalent U(VI) to a tetravalent form U(IV), such as the uraninite (UO_2) precipitate, is a promising technology to remediate uranium-contaminated groundwater and soil. Field studies revealed that U(VI) can be reduced and immobilized simply by amending the contaminated site with organic substrates, such as glucose or acetate. However, complete reduction of U(VI) to U(IV) may not be always possible due to the nature of the chemical form, the mineralogical association of uranium, and the conditions favorable for mediating the biogeochemical processes in contaminated sites.

Stabilizing uranium in wastes by enhancing anaerobic microbial activity was demonstrated by exploiting the unique metabolic capabilities of the dual-action anaerobic bacterium *Clostridium* sp. to solubilize and precipitate uranium in a sludge and sediment sample (Francis et al., 1991b). Sludge was collected from a uranium-process waste stream after the bio-denitrification of nitric-acid uranium waste water, and the sediment from a contaminated pond that received uranium-process waste-water at the Y-12 Plant at Oak

Ridge, TN. Table 1 shows the chemical characteristics of the sediment and sludge samples. Both samples contained varying levels of the major elements, Al, Ca, Fe, Mg, K, and Na, and toxic metals, As, Cd, Cr, Co, Cu, Pb, Mn, Hg, Ni, U, and Zn. The concentrations of uranium in the sediment- and sludge-samples respectively were 920 ppm, and 3100 ppm. The sediment contained high levels of Cr, Cu, Mn, Hg, and Zn. In comparison, the sludge was high in Ca, low in Fe and Mn, and contained higher amounts of Cd, Cr, Pb, and Ni. In addition to those elements listed in Table 1, analysis of the sediment sample by x-ray fluorescence showed the presence of titanium, gallium, bromine, strontium, rubidium, yttrium, and zirconium. Selenium, a common element present in waste from uranium mining, was not detected in these samples. The sediment had a high ash content, and appreciable amounts of organic carbon and nitrogen; x-ray diffraction analysis showed high levels of SiO_2. The sludge was low in organic carbon and nitrogen but high in ash and sulfate; the latter resulted from adding sulfuric acid and ferric sulfate in the waste-treatment process.

The mineralogical association of cadmium, chromium, copper, manganese, nickel, lead, uranium, and zinc in the sludge and sediment was determined by a selective extraction procedure (Tessier et al., 1979, Francis et al., 1991a); Figure 1 shows these associations of uranium in the sludge and sediment. The inert (predominantly silicates) and the organic fractions respectively contained 301- and 293-µg/g of uranium, and the Fe-Mn- oxide fraction contained 162 µg uranium/g dry wt. Nearly half of the total uranium (1600 µg) in the sludge was associated with the carbonate fraction (Figure 1). The association of uranium with other fractions was as follows: Exchangeable, 400 µg; iron oxide, 216 µg; organic, 516 µg; and, inert, 80 µg/g dry wt. Comparing the total uranium obtained by digesting the entire sample with the sum of the selective extractions showed good agreement within ±10% (±1 SEM).

Clostridia are ubiquitous in soils, sediments, and wastes, and could be very useful in pretreating and stabilizing uranium in radioactive wastes. To determine the anaerobic microbial transformations of uranium sludge and sediment, samples were incubated anaerobically with and without nutrients in an N_2 atmosphere. The unamended samples showed neither significant microbial activity nor produced organic-acid metabolic products. However, the amended samples showed an increase in total gas, CO_2, H_2, CH_4, and organic acids; pH was lowered by about 2.5 units. This change was due to the generation of organic-acid metabolites from glucose fermentation. The organic acids were acetic-, butyric-, propionic-, formic-, pyruvic-, lactic-, isobutyric-, valeric-, and isocaproic- acids. A significant amount of gas was produced due to glucose fermentation by anaerobic bacteria, as well as from the dissolution by the organic acids of $CaCO_3$ in the sludge. A decrease in sulfate concentration was observed only in amended samples.

This treatment process removed a large fraction of soluble non-toxic metals, such as Ca, K, Mg, Mn^{2+}, Na, and Fe^{2+}, and enriched and stabilized Cd, Cr, Cu, Ni, Pb, U, and Zn with the remaining solid phase due to the direct- and indirect-actions of the bacteria (Francis, et al. 1991a). Metals associated with the exchangeable-, carbonate-, and iron oxide-fractions were solubilized indirectly via the production of organic-acid metabolites, whereas dissolution of iron oxides and metals co-precipitated with iron oxides was due to direct enzymatic reduction of iron. The uranyl ion associated with the exchangeable-, carbonate-, and iron-oxide-fractions was released into solution by direct- and indirect-actions of the bacteria, and subsequently, was reduced enzymatically to insoluble U(IV) (Figures 2-4). X-ray absorption near edge spectroscopic (XANES) analysis of uranium in the untreated (control), the treated sludge and sediment samples showed the partial reduction of U(VI) → U(IV) in the sludge, and its complete reduction in the sediment (Figure 4). Uranium was predominantly associated with the carbonate fraction, and to a lesser extent, with the oxide-,

organic-, and inert-fractions; after microbial activity, its concentration increased for all three fractions. Analysis of the mineralogical association of the metals in the wastes after microbiological action showed that many of the metals were redistributed with stable mineral phases, such as the organic- and silicate-fractions (Figure 5). An overall reduction in the volume and mass of the waste after microbial action was apparent due to the removal of the bulk soluble non-toxic components from the waste, such as Ca, Na, and Fe.

This biotreatment process (Figure 6) can be applied to mixed wastes containing radioactive elements and toxic metals generated from defense-, energy-, and industrial-operations wastes to chemically convert the radionuclides and toxic metals to more stable forms. Reducing the mass of the wastes means that more material can be stored or disposed of, can be handled easier, and can be transported. It also facilitates changing the radionuclides and toxic metals to more stable forms so that the material can be processed chemically for disposal in shallow- or deep-geological formations.

20.6.1 Remobilization of Uranium. Under anaerobic conditions, some host metal (hydr)oxides (e.g. Mn^{4+} and Fe^{3+}) can be reduced either chemically or enzymatically to soluble forms. Microbially mediated reductions play an important role in the biogeochemical cycles of metals, and can also lead to the remobilization of sorbed metals, such as Cd, Cr, Ni, Pb, and Zn to iron- and manganese-oxides (Francis and Dodge 1990). Further, the remobilization of bio-reduced U(IV) can be reoxidized to U(VI) by Fe-(III) and Mn(IV) with the formation soluble uranyl carbonate.

Although the U(IV) solids and U(VI)-bearing Fe or Al minerals are relatively stable, the presence of organic complexing agents and dissolved organic matter also may affect the long-term stability of uranium in contaminated sites. The bio-reduced U(IV) precipitates can be oxidized and then subjected to dissolution or remobilization by various oxidants, such as dissolved oxygen, ferric iron, and nitrate, or through complexation by organic ligands.

Naturally occurring organic ligands, such as siderophores, citrate, phthalates, humic substances, and synthetic chelating agents, such as ethylenediaminetetraacetate (EDTA) can mobilize both reduced U(IV) and oxidized U(VI) by forming soluble complexes. Remobilization of biologically immobilized U(IV) by naturally occurring organic ligands is a major concern in the long-term stability of uranium following in situ remediation (Gu et al., 2005; Luo and Gu, 2009, 2011; Francis and Dodge, 2008).

20.7. *Ex-situ* remediation of uranium-contaminated soils, sediments, and wastes

For decontamination, radionuclides must be removed and recovered from the contaminated site, so that the site is restored. Various soil-washing techniques were developed, including physical methods, such as wet-screening, attrition-scrubbing, or chemical methods consisting of treating with organic and inorganic acids, salts, bases, and chelating agents. For example, the following chemicals have been used to extract radionuclides and toxic metals: Nitric acid, hydrochloric acid, phosphoric acid, sulfuric acid, sodium carbonate, ammonium carbonate, sodium hydroxide, oxalic acid, citric acid, EDTA, and DTPA. Many of the inorganic chemicals used are corrosive, which irreparably damages the soil. Furthermore, all chemical extraction methods generate secondary waste streams that entail additional problems of hazardous waste disposal.

20.7.1 Bicarbonate extraction. Carbonate extraction of soils is an attractive procedure because U(VI) forms a very stable complex with carbonate $[UO_2(CO_3^{-2})_3^{-4}]$, and carbonate generates a waste stream with lower concentrations of secondary soil constituents consisting of iron, aluminum, calcium, and silica than do acid extractions. Bicarbonate is environmentally benign and has no deleterious effects on the soil's structure.

A process for remediating and recovering uranium from contaminated soils was developed using bicarbonate extraction (Philips et al., 1995). It consists of two steps: (i)

Extraction of uranium from contaminated soils using bicarbonate, and, (ii) reductive precipitation of U(VI) in bicarbonate extracts by sulfate-reducing bacteria *Desulfovibrio desulfuricans*. The extraction efficiency of bicarbonate ranged from 20 to 94% of the nitric-acid-extractable uranium. However, the kinetics of uranium extraction by bicarbonate varied among ores, acid tailings, mixed tailings, and alkaline tailings. In the second step, uranium in the bicarbonate extracts was recovered as uraninite particles by dissimilatory microbial reduction of soluble U(VI)-carbonate complexes. Microbial reduction is more effective in removing uranium from bicarbonate extracts than are physiochemical methods, such as ion exchange. The precipitated uranium recovered as uraninite is either reused or disposed of as waste. The bicarbonate extract is recycled to retrieve more uranium.

20.7.2 Citric-acid Extraction. Citrate is a naturally occurring organic complexing agent well known for its ability to form strong complexes with uranium (Rajan and Martell 1965). It also is known to effectively remove metals from soils and wastes (Jackson et al. 1986). It is environmentally friendly, exhibits relatively consistent removal efficiency, and is cost-effective. Citric acid forms different types of complexes with the transition metals and actinides, and has been used to extract plutonium from contaminated soils (Nishita, 1977), to decontaminate the components of nuclear reactors, (U.S. Patent Nos. 4,839,1000; 4,729,855; 4,460,500; 4,587,043; 4,537,666; 3,664,870, and 3,103,909), and to extract metals and radionuclides, such as arsenic, barium, cadmium, cesium cobalt, copper, chromium lead, nickel, zinc, strontium, thorium, and uranium, from contaminated soils, wastes, and municipal solid-waste incinerator ash (Francis and Dodge, 1992, 1994a; Francis et al., 1993; Peters et al., 1995).

A bi-sequential extraction procedure using citric acid in combination with dithionite followed by ammonium carbonate extraction with potassium permanganate removed uranium from at levels of 450-540 mg/kg, to levels <50 mg/kg in Fernald soils. The first extraction is

based on the citrate-bicarbonate-dithionite (CBD) procedure, developed by Jackson et al. (1986) to remove sesquioxides from layer silicates. The basic principle is reducing the ferric oxides to ferrous forms, which weakens the crystalline characteristics of the mineral phase, and results in the reductive dissolution of ferric iron. Citrate is used to chelate the ferrous iron and prevent its precipitation. The bicarbonate maintains a pH (7.3) that favors a strong reducing environment created by adding dithionite, a strong reductant. The higher pH of this extraction environment also removes significantly less calcium and magnesium from dolomite and calcite (carbonate minerals) than does citric acid or sulfuric acid. The second extraction (that uses ammonium carbonate and potassium permanganate) is intended to oxidize the remaining $U(IV)$ to $U(VI)$ to form the strong uranyl carbonate complex (Francis, et al., 1993). In 4-h batch-type stirred reactor tests, citric acid extracted ~95% of uranium from Fernald's storage pad soil, and ~60% from incinerator site soil. The extraction of uranium from both soils by citric acid was highly dependent on pH; the most effective extraction occurred at a pH value of 4.

Citric acid is a good extractant because at low pH values it promotes the dissolution of carbonate minerals and iron and aluminum sesquioxide coatings on soil particles. i.e., the two mineral phases may act as contamination sites for uranium in soils. Consequently, large quantities of calcium, magnesium, iron, and aluminum are present in citric-acid effluents, which complicate the removal of uranium more difficult and the disposal of the waste stream more voluminous and complex than extracting with carbonate.

Citric acid has several advantages over sulfuric acid leaching: (1) It biodegrades rapidly to carbon dioxide and water, making the treatment and disposal of the effluent more environmentally benign; (2) it is inexpensive, and even may be obtained as an industrial waste-product; and, (3) it offers a buffered system in contrast to sulfuric acid wherein the pH of the extraction suspension varies widely as the carbonates are neutralized.

20.7.3 Remediation of uranium contaminated soils and wastes by citric acid.

In this process, uranium and toxic metals are extracted from wastes or contaminated soils with the complexing agent, citric acid. Then, the citric-acid extract first is biodegraded to recover the toxic metals, followed by photochemical degradation of the uranium citrate complex that is recalcitrant to biodegradation. The toxic metals and uranium are recovered in separate fractions for recycling or disposal. Using this combined chemical and microbiological treatment is more efficient than present methods and should result in considerable savings in the costs of clean-up and disposal.

20.7.3.1   *Metal citrate complexes*. Citric acid, a natural organic compound, is a multidentate ligand that forms stable complexes with various metal ions (Glusker, 1980; Rajan and Martel 1965). The type of complexes it forms is shown in Figure 7. The biodegradation of these complexes depends on the type of metal complex formed with citrate; bidentate ones readily are biodegraded whereas the tridentate- and binuclear-complexes are recalcitrant (Francis et al., 1992).

20.7.3.2   *Biodegradation of metal citrate complexes*. The rate and extent of biodegradation of several metal-citrate complexes by microorganisms varies. For example, *Pseudomonas pseudoalcaligenes* degraded Mg-citrate at a much lower rate than Ca-, Fe(III), and Al(III)-citrate (Madsen and Alexander, 1985). Studies with a *Klebsiella sp.* showed that citric acid and Mg-citrate were readily degraded, whereas Cd-, Cu-, and Zn-citrate were resistant (Brynhildsen and Rosswall, 1989). Both studies also revealed that metal toxicity was not responsible for the lack of, or the lower rate of degradation of certain metal-citrate complexes but offered no other explanation. Biodegradation studies with *Pseudomonas fluorescens* showed that bidentate complexes of Fe(III)-, Ni-, and Zn-citrate were readily biodegraded, whereas complexes involving the hydroxyl group of citric acid, the tridentate Al-, Cd- and Cu-citrate complexes, and the binuclear U-citrate complex were not (Francis et

al., 1992) (Figure 8). The presence of the free hydroxyl group of citric acid is the key determinant in effecting such biodegradation. The lack of degradation was not due to their toxicity, but was limited by the transport and/or the bacterial metabolism of the complex. No relationship was observed between biodegradability and stability of the complexes. The tridentate Fe(II)- citrate complex, although recalcitrant, was readily biodegraded after oxidation and hydrolysis to the bidentate Fe(III)-citrate form, denoting the structure-function relationship in the metabolism of the complex (Francis and Dodge, 1993). Although uranyl citrate was recalcitrant to biodegradation it was readily photodegraded by light (Figure 9).

20.7.3.3 *The Citric Acid Process.* Although citric acid is an effective chelating agent for removing metals and radionuclides from contaminated soils, the ultimate disposal of the citric-acid-metal extract is a concern. Francis and Dodge (1994) developed a process to recover the metals and radionuclides from the extract (Figure 10), in which the extract undergoes microbiological degradation, followed by photochemical degradation under aerobic conditions. Several metal-citrate complexes are readily biodegraded, and the metals are recovered in a concentrated form, along with the bacterial biomass. Uranium that forms a binuclear complex with citric acid is not biodegraded. The supernatant containing this complex is separated, and upon exposure to light, it rapidly degrades forming an insoluble, stable polymeric form of uranium. Uranium is recovered as a precipitate ($UO_3 \cdot 2H_2O$) in a concentrated form for recycling or for disposal (Figures 9 and 10). This treatment process, unlike others which use alkaline or acidic reagents, does not create additional hazardous wastes for disposal, and causes little damage to the soil that then can be returned to normal use.

20.7.3.4 *Extraction of radionuclides and metals from soils and wastes*. Uranium-contaminated soil samples were obtained from the Fernald site in Ohio, the RMI site, Ahstabula, Ohio, and uranium-contaminated sediment and sludge samples from the West

End Treatment Facility, at the U.S. Department of Energy, Oak Ridge Y-12 Plant, Oak Ridge, TN.; they were analyzed for total uranium, its mineralogical association, and its speciation. The concentrations of U in the soils varied from 473- to 1880-ppm (Table 2). RMI soils also contained Tc.

Ten grams of soil or sludge from the Oak Ridge Y-12 Plant containing uranium were extracted with 100 ml of 0.4 M citric acid for five hours in the dark. The citric-acid extract and the solids were separated, and analyzed for metals. In this sample, aluminum, beryllium, cobalt, chromium, manganese, nickel, antimony, tin, zinc, and zirconium were extracted with >50% efficiency; uranium and thorium were extracted with 87% and 94% efficiency, respectively. Silver, copper, lead, and vanadium were not extracted, probably due to the type of their association with stable mineral phases (Francis et al., 1998). For example, copper predominantly was associated with the organic fraction and a small amount with the iron oxide- and inert-fractions. Table 2 lists the efficiency of extraction of uranium from Oak Ridge Y-12 plant sludge and sediment, and various soils from the Fernald and RMI sites, Ohio.

20.7.3.5   *Biodegradation of citric-acid extracts containing metals.* Citric-acid extracts were amended with 0.1% NH_4Cl, K_2HPO_4, and KH_2PO_4, then the pH was adjusted to 6.5 and finally, the extract was inoculated with a culture of *Pseudomonas fluorescens* (ATCC #55241). The bacteria degraded citric acid at a rate of 0.5-0.7 mM per hour; there was little change in concentration of uranium, suggesting that the uranium-citrate complex was not biodegraded (Figures 11A-D). Other metals present in the extract, viz Al, Ba,Ca, Fe, Cu, Mn, Ni, Pb, Sr, Ti, and Zn, were removed from it through the biodegradation of their respective citrate complexes, or precipitated from solution due to changes in the pH as result of citrate degradation and were recovered along with the biomass (Figure12).

20.7.3.6   Photodegradation of uranium citrate extracts. The pH of the supernate from the biodegradation treatment primarily containing the uranium-citrate complex was adjusted to pH 3.5 with HCI, and then exposed under high-output fluorescent growth lights to degrade the complex and recover uranium (Figure 13). The uranium precipitated out of solution as a polymer soon after it was exposed to light; after 50 hours, ~ 85% of the uranium was removed. The uranium precipitate was identified as ($UO_3·2H_2O$) by x-ray photoelectron spectroscopy (XPS), and by x-ray absorption near-edge spectroscopy (XANES) (Figure 14). The uranium precipitate was almost insoluble at near neutral pH, but soluble in acidic pH (<3.5). The solids remaining after extraction with citric acid were washed with deionized water, and dried in an oven overnight at 105^oC to determine the weight loss due to removing the citric acid. Extracting the metals from the waste reduced its weight by 47%.

These results show that (i) uranium was extracted from the mixed waste with >85% efficiency using 0.4 M citric acid; (ii) other metals such as chromium, cobalt, manganese, nickel, strontium, thorium, zinc, and zirconium also were extracted; (iii) the uncomplexed excess citric-acid and several metal citrate complexes (Co, Ni, Zn and, Zn) except the binuclear uranium- citrate complex, readily were biodegraded by *Pseudomonas fluorescens*, and the metals were recovered with the bacterial biomass; and, (iv) the uranium citrate complex was photodegraded, allowing the uranium to form a polymer that was recovered as a concentrated solid. Thus, the toxic metals and uranium are recovered in a concentrated form for recycling or for disposal.

This process has significant potential applications (i) it can be applied to a variety of materials and waste forms; (ii) mixed waste is separated into radioactive- and hazardous-wastes; (iii) uranium is separated from the toxic metals and recovered for recycling or disposal; (iv) it does not generate secondary-waste streams; (v) it causes little damage to the

soil; and, (vi) environmentally and economically important metals are removed in a concentrated form. Using combined chemical-, photochemical-, and microbiological-treatments of contaminated materials will be more efficient than the present methods and result in considerable savings in the costs of clean-up and disposal.

20.7.4. *In-situ* leaching (ISL)

In-situ soil flushing is used to mobilize metals by leaching the contaminants from soils so that they can be extracted without excavating the contaminated materials. An aqueous extracting solution is injected into, or sprayed onto the contaminated area to solubilize and mobilize the contaminants. The extractant can be applied by surface flooding, sprinklers, leach fields, vertical- or horizontal-injection wells, basin infiltration systems, or trench infiltration systems. After coming into contact with the contaminated material, the extractant solution is collected using pump-and-treat methods for its disposal or its treatment and reuse. Extracting agents used for *in-situ* soil flushing and washing include acids/bases, chelating agents (oxalate, citrate, EDTA, DTPA), oxidizing/reducing agents, and surfactants/cosolvents. Water alone can be used to remove water-soluble contaminants. The applicability of in-situ soil flushing technologies to contaminated sites will depend largely on site-specific properties, such as hydraulic conductivity, that influence the ability to contact the extractant with contaminants, and to effectively recover the flushing solution with collection wells.

20.7.4.1 In-situ leaching (ISL) of uranium. Conventional mining involves removing mineralized rock (ore) from the ground, breaking it up, and treating it to remove the minerals. *In-situ* leaching (ISL), also known as solution mining, or *in-situ* recovery (ISR), involves leaving the ore where it is in the ground, and recovering the minerals from it by dissolving them, and then pumping the pregnant solution to the surface where the minerals are recovered. Consequently, there is little surface disturbance and no tailings or waste rock

are generated. However, the ore body must be permeable to the liquids used, and located such that the liquids do not contaminate the groundwater away from the ore body. ISL can also be applied to other minerals, such as copper and gold, for uranium- and other radionuclide-contaminated soils. ISL techniques were developed where it is controllable, safe, and environmentally benign method of mining, operating under strict operational- and regulatory-controls. Due to the low capital costs (relative to conventional mining), it often proves to be an effective method of mining low-grade uranium deposits.

20.7.4.2 **Uranium recovery.** The native groundwater from the host aquifer initially is extracted by submersible pumps, after which uranium complexing reagents (acid or alkaline) and an oxidant (hydrogen peroxide or oxygen) are injected into the well field. The leach liquors pass through the ore to oxidize and dissolve the uranium minerals *in-situ*. Depending on the type of leaching environment, the uranium will be complexed as uranyl sulphate, predominantly $UO_2(SO_4)_3^{4-}$ in acid leach conditions, or a uranyl carbonate, predominantly $UO_2(CO_3)_3^{4-}$, in a carbonate leach system. This then is precipitated with an alkali, e.g., as sodium- or magnesium-diuranate. In either case, the pregnant solution from the production wells is pumped to the treatment plant where the uranium is recovered in a resin/polymer ion-exchange system, or liquid-ion exchange one (*World Nuclear Association, U K*).

20.8 Plutonium biotransformation and remediation studies

Plutonium (Pu) occurs in several oxidations states, i.e., Pu(III), Pu(IV), Pu(V), and Pu(VI) in the environment, and its solution chemistry is very complex. However, Pu(IV) is the predominant, most stable form in contaminated soils. Due to high ionic charge, it can undergo hydrolysis and convert to a polymeric form at pH>2. Although Pu is considered relatively immobile, its transport was observed at several DOE sites, even at very low

concentrations. The chemical speciation of Pu can be influenced by the soil's pH, redox conditions, organic content, mineralogy, and microbial activities. The chemical form of Pu varies in contaminated sites, and is dependent on environmental conditions prevailing there, and upon the waste stream. $PuO_2(s)$ was the predominant form at Rocky Flats, Colorado (Clark et al., 2006), whereas Pu was associated with mineral- or organic-colloids at the Nevada Test Site and Rocky Flats site (Kersting et al., 1999; Xu et al., 2008). Microbial activities affect redox conditions, and thus, the oxidation state of Pu in the environment (Neu et al., 2005). Information is limited on the influence of microbes on the solubility of Pu (Deo and Rittmann 2012; Deo et al., 2011). The bacterial reduction of Pu(VI) and Pu(V) to Pu(IV) was shown to immobilize soluble Pu. The microbial reduction of Pu(VI/V) potentially can produce a highly insoluble form of Pu(IV). However, amorphous Pu(IV) can be further reduced anaerobically to the more soluble Pu(III) by *Clostridium* sp. (Francis et al., 2008); by *Bacillus subtilis* in the presence of the chelating agent NTA(Rusin et al 1994); and by *Geobacter metallireducens* and *Shewanella oneidensis*with EDTA (Boukhalfa et al., 2007).

Francis et al., (2007) detailed the aerobic biotransformation of Pu(IV)-citrate by *Pseudomonas fluorescens,* finding that citrate was degraded rapidly whilst a polymeric form of Pu(IV) formed. Adding Pu(VI) to *Bacillus subtilis* cells and kaolinite clay engendered the formation and association of Pu(IV) predominantly with bacterial cells. The reductive transformation of Pu(VI) to Pu(IV) was observed only in the presence of *Bacillus subtilis* cells; kaolinite clay alone did not cause any changes in the oxidation state of Pu. The remobilization of Pu and other radionuclides was observed in Pu-contaminated soils from Nevada Test Site caused by enhanced microbial activity under aerobic- and anaerobic-conditions and affected the concentration of Pu in the solution phase (Francis et al., 2007).

20.8.1 *Effect of microbial activity on Pu mobilization from contaminated soils.*

Characterization of Pu at contaminated sites shows that its environmental form varies

according to the site and the waste stream. For example, at Rocky Flats, CO, the predominant form appears to be as $PuO_2(s)$, while, at the Nevada Test Site (NTS), Pu was associated with mineral oxides; at Oak Ridge, TN it is associated with organic matter. Pu generally is considered to be relatively immobile; however, its transport to varying distances in low concentrations as colloids was observed at Rocky Flats, Los Alamos National Laboratory (LANL), and the NTS. Plutonium in surface waters at the Rocky Flats site was associated with organic macromolecules. Studies with Pu-contaminated soils show that Pu and other radionuclides are remobilized due to enhanced aerobic- or anaerobic-microbial activity (*Francis and Dodge, unpublished results*). Several bacteria and fungi grown in the presence of Pu produced extracellular Pu complexes that raised the concentration of Pu in soil-column eluates relative to that of the controls. Elution through soil effectively removed positively charged Pu complexes. The increased mobility of Pu in soil reflected the formation of neutral- and negatively charged-Pu complexes. In the presence of known microbial metabolites and synthetic ligands (i.e., DTPA, EDTA, and EDDHA), Pu(VI) was reduced to Pu(IV) before complexation, suggesting that the latter valence state would be the dominant one associated with organic complexes in soils.

20.8.2 Characterization Pu in contaminated Soil. Plutonium contaminated soil (HP-11) obtained from Area 11 soil of the Double Track test shot area at the NTS had a gross activity of 50 nCi/g. The individual alpha components of the total activity were determined and are given in Table 3. The beta-emitter Pu-241 also was detected but not quantified. The minerals present in the soil were determined using μ-X-ray diffraction on beamline X7A at the National Synchrotron Light Source (NSLS); the predominant ones were various forms of iron oxides and aluminosilicates. The uranium was present in the soil as hexavalent form associated with the minerals schoepite ($UO_3 \cdot 2H_2O$) and liebigite ($Ca_2UO_2(CO_3)_3 \cdot 11H_2O$).

20.8.3 Mobilization of Pu, Am, and U in NTS soil due microbial activity.

Figure 15 illustrates the effect of bacterial activity on the mobilization of radionuclides in NTS soil. Under anaerobic conditions, glucose was metabolized with a decrease in pH to 4.8 and an increase in alpha activity in solution. However, the appearance of the actinide in solution was concomitant with the decrease in pH (Figure 15). In addition, the alkalinity of the soil suggests that its mobilization may be due to the dissolution of carbonate species present in the soil.

Adding citric acid to the soil showed the metabolism of citric acid and an increase in both alpha- and beta-activity in solution. However, with time there was sudden decrease in alpha and beta activity (data not shown) that coincided with the complete utilization of citric acid by the bacteria. There was only a slight decrease in pH to 7.7 in the bacterially active sample. This observation suggests that citric acid forms a soluble complex with the actinide, and that its removal due to bacterial metabolism releases the actinide that then is precipitated. These results also suggest that the type of carbon source will have a different effect on microbial mobilization of actinides.

20.9   Summary

Microorganisms play a major role in the environmental mobilization and immobilization of radionuclides. Such microbial processes are being exploited to extract or stabilize radionuclides contaminated soils. However, the biochemical mechanisms of biotransformation of the various chemical forms of radionuclides found in contaminated soils are not fully understood, and consequently, in-situ or ex-situ bioremediation of contaminated soils has not been deployed. Based on our current understanding of uranium biogeochemistry, it appears that microbial reductive-precipitation of uranium is an attractive *in-situ* bioremediation process that can be developed further provided that reducing conditions can be maintained to limit the re-oxidation and re-mobilization of sequestered U. Alternatively,

extracting uranium from contaminated soils by carbonate or citric acid is a potential promising *ex-situ* process that was validated in laboratory- and batch- studies. The microbially induced calcite precipitation of strontium via bacterial ureolysis as an in-situ process was demonstrated in laboratory- and simulated field-scale studies. The short half-life of ^{90}Sr (29 years) and the long-term stability of calcite make this co-precipitation process very attractive to sequester the radionuclide that it would permit sufficient time for radioactivity to decay.

Acknowledgments

20.10   Suggested Readings

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Table I. Characterization of Oak Ridge Sediment and Sludge Samples (Francis et al 1991b; *manuscript in preparation***).**

Constituents	Sediment	Sludge
Physical, %		
moisture	60.8 ± 0.1*	56.7 ± 0.4
ash	79.8 ± 0.0	65.3 ± 0.0
Chemical, % dry weight		
carbon	12.0 ± 0.1	1.35 ± 0.0
nitrogen	0.44 ± 0.0	0.12 ± 0.0
sulfate sulfur	0.99 ± 0.03	2.57 ± 0.05
Major metals, % dry weight		
aluminum	5.34 ± 1.8	5.21 ± 0.02
calcium	3.21 ± 0.01	24.1 ± 1.2
iron	4.66 ± 0.17	0.50 ± 0.01
magnesium	1.05 ± 0.10	1.30 ± 0.09
potassium	1.19 ± 0.02	0.05 ± 0.0
sodium	0.09 ± 0.01	1.87 ± 0.04
Toxic metals, mg.gdw^{-1}		
arsenic	19.8 ± 2.3	1.3 ± 0.0
cadmium	13.3 ± 0.1	93.5 ± 0.3
chromium	421± 6	396 ± 9
cobalt	81.3 ± 12.1	38.7 ± 10.5
copper	394 ± 2	371 ± 1
lead	195 ± 12	267 ± 18
manganese	629 ± 9	244 ± 4
mercury	342 ± 4	10.6 ± 1.1
nickel	188 ± 2	1260 ± 5
selenium	<1.0	<1.0
uranium	647 ± 42	2700 ± 200
zinc	1030 ± 40	1000 ± 30

* ± 1 standard error of the mean.

Table 2. Analyses of contaminated soils and wastes and extraction of uranium by citric acid (Francis and Dodge (1998; *unpublished results*)**.**

Sample	Technetium (pCi/g)	Uranium (ppm)	Citric acid (M)	Extracted (%)
Ashtabula, OH				
RMI #27117	120±7*	1880±20	0.5	83
RMI #27227	109±15	698±19	0.5	64
RMI #27230	144±47	725±38	0.5	69
FERMCO, OH				
Soil # 4 02585	NA	473±10	0.5	86
Storage pad soil**	NA	450	0.4	99
Y-12 Plant, Oak Ridge, TN				
Sludge	NA	2410	0.4	87
Pond Sediment	NA	920	0.1	33

*   = ± 1 standard error of the mean.
**Data from C.W. Francis et al. 1993.
NA = not analyzed.

Table 3. Alpha activity and concentration in NTS soil (Francis and Dodge, *manuscript in preparation*)

Isotope	NTS Soil	
	(nCi/g)	(μg/g)
U 233/234	< 0.18	< 6.1 x 10^{-2}
U 235/236	< 0.15	< 3.6 x 10^{1}
U 238	< 0.14	<4.2 x 10^{2}
Am 241	3.8 ± 0.7	1.1 x 10^{-3}
Pu 238	0.44 ± 0.02	2.6 x 10^{-5}
Pu 239/240	69.2 ± 6.5	1.1 x 10^{-1}
Total	<75	ND

ND = not determined
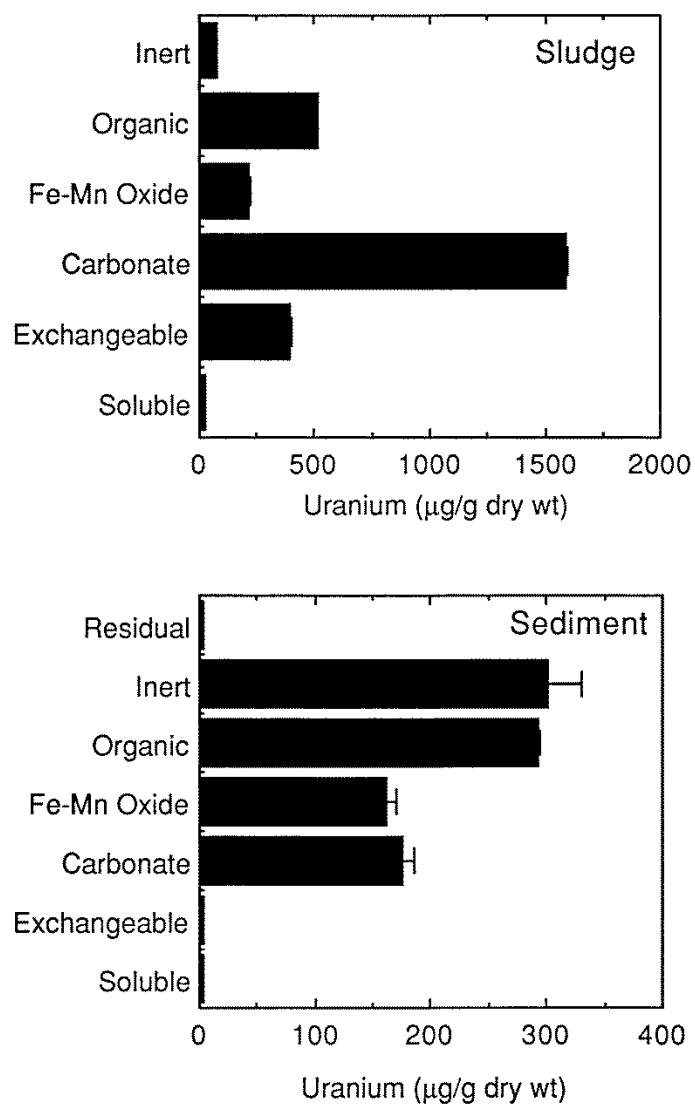
Figure 1. Mineralogical association of uranium in Y-12 sludge and sediment (Francis et al., 1991b; *manuscript in preparation*).
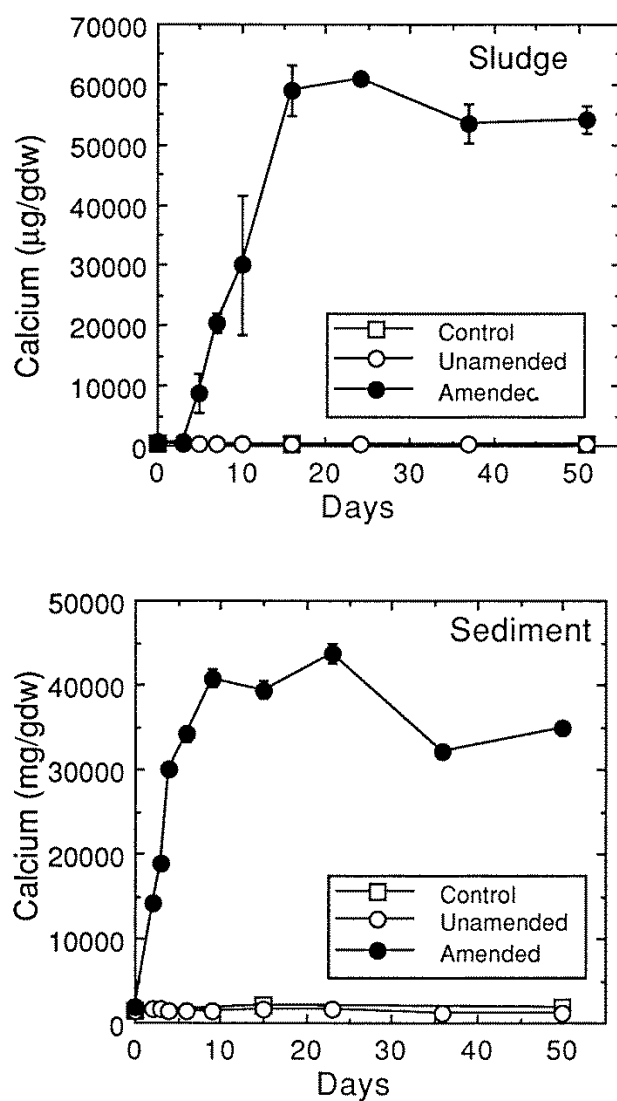
Figure 2. Dissolution of calcium from Y-12 sludge and sediment by indirect action of the bacteria due to production of organic acids and lowering the pH of the medium (Francis et al., 1991b; *manuscript in preparation*).
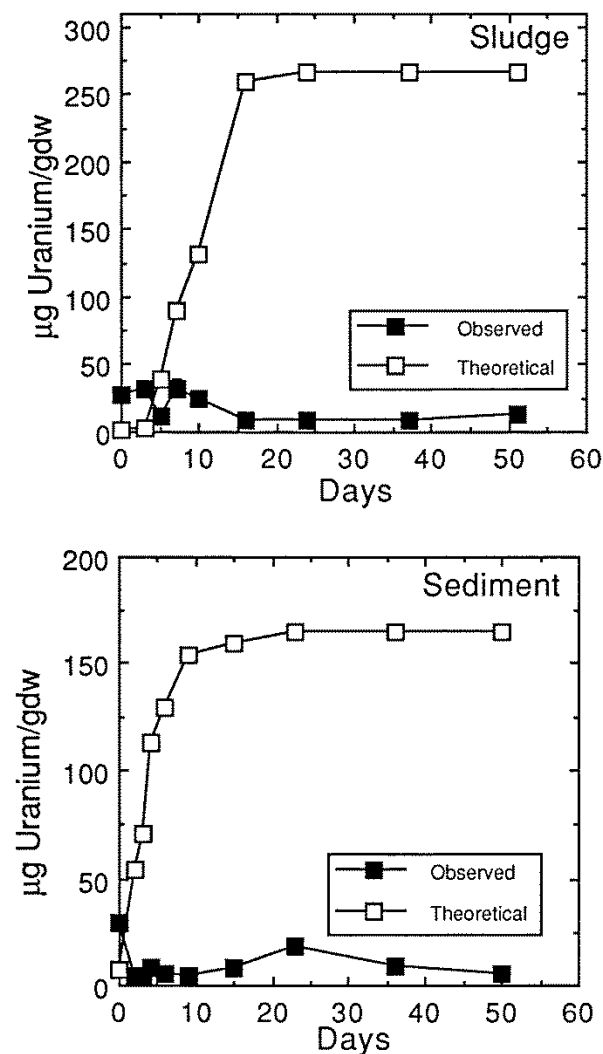
Figure 3. Dissolution and precipitation of uranium from Y-12 sludge and sediment. U(VI) associated with carbonate fraction is solubilized due to indirect action; with the iron oxide due to reductive dissolution of Fe(III) to Fe(II) and the released U(VI) is reduced to U(IV) by enzymatic action (Francis et al., 1991b; *manuscript in preparation*).
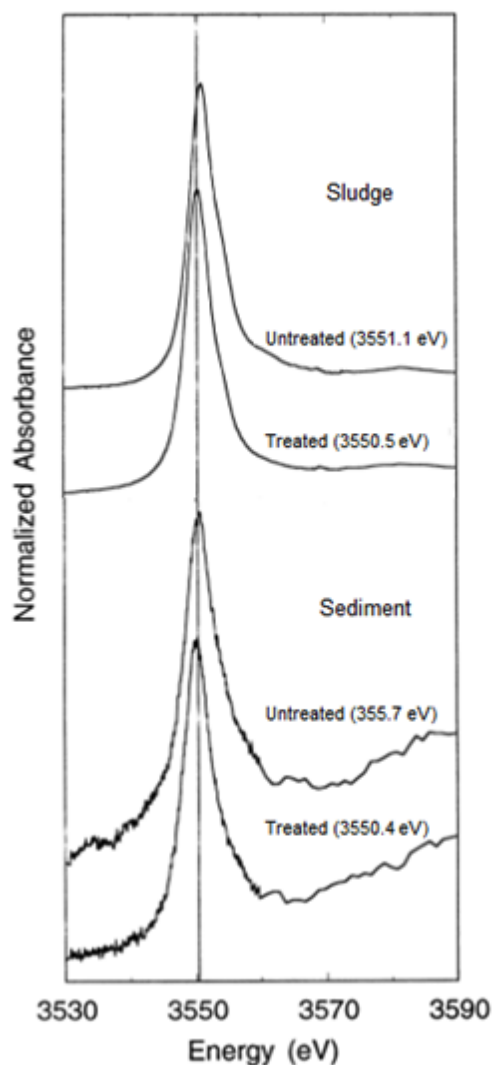
Figure 4. XANES analyses of the sludge and sediment from the Y-12 Plant, Oak Ridge, TN at the Mv edge before and after bacterial activity. The sludge contained 2070 of ppm uranium and the absorption maximum for the untreated sample was 3551.1 eV, indicating that the uranium was predominantly in the hexavalent form. After bacterial treatment there was a shift in the absorption maximum to 3550.5 eV, which is slightly higher than tetravalent uranium (3550.4 eV), but much less than U(VI) indicating reduction to U(IV). The sediment contained 647 ppm of uranium and the untreated sample showed an absorption maximum at 3550.7 eV, which corresponds to a mixture of U(IV) and U(VI). The sediment was rich in organic matter and reducing. In the bacterially treated sample the peak shifted to 3550.4 eV, which was identical to U(IV), indicating that the uranium was reduced to the tetravalent form (Francis et al. *manuscript in preparation*).
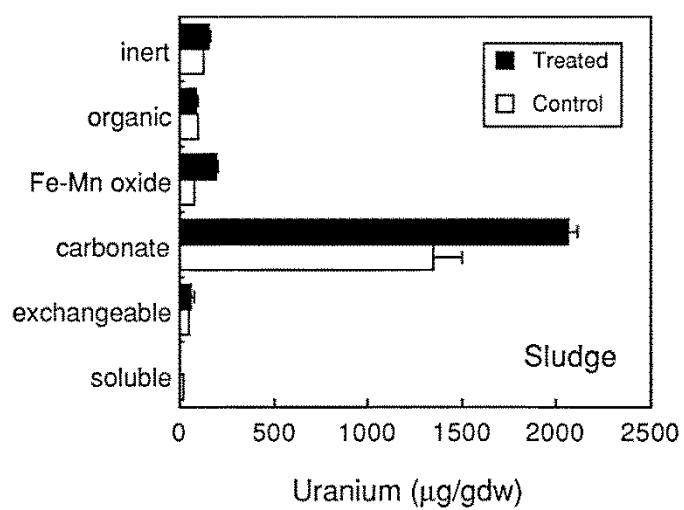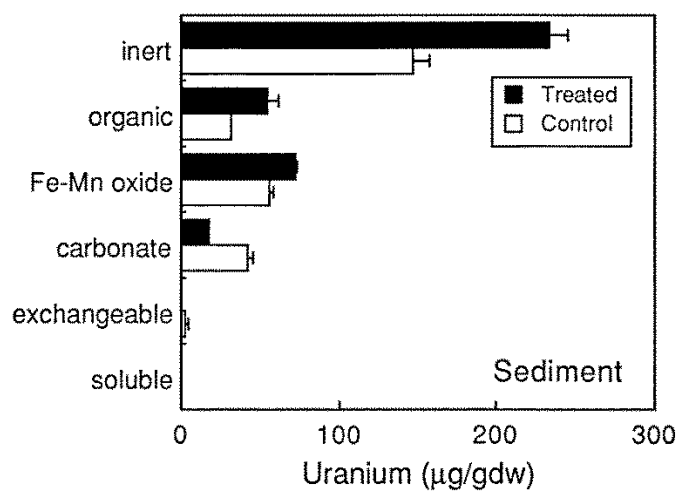
Figure 5. Mineralogical association of uranium in sediment and sludge before and after anaerobic microbial action (Francis et al., 1991b; *manuscript in preparation*).
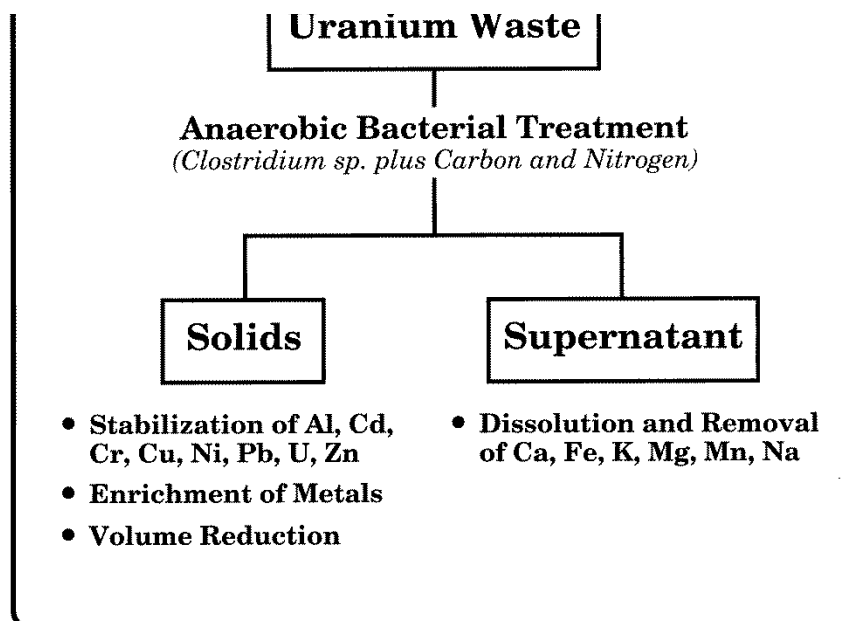
Figure 6. Stabilization of uranium in wastes, soils and sediments by anaerobic bacterial treatment. Substantial amounts of bulk non-toxic elements Ca, Fe, K, Mg, Mn, and Na were solubilized from the waste, reducing the mass by ~20% in batch studies. Further reduction can be achieved by optimization of process parameters. Stabilization and reducing the volume reduces disposal costs (Francis 1991b; Francis 1994).
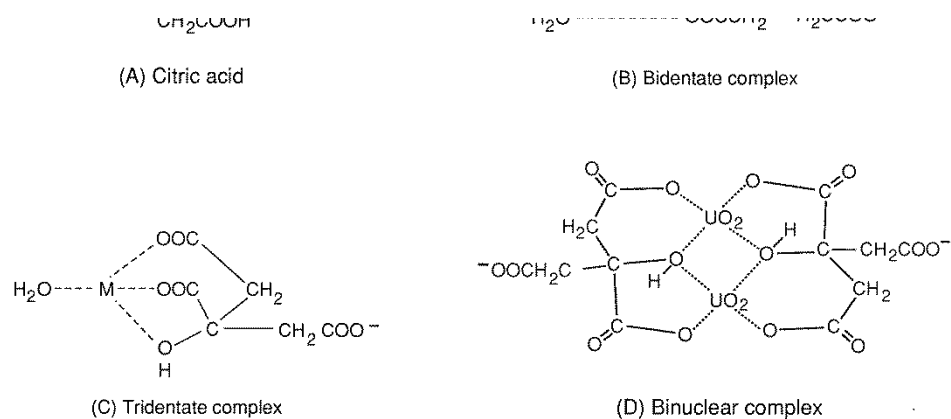
Figure 7. Metal citrate complexes. Citric acid a natural organic compound capable forming different types of complexes with metals. It forms bidentate-, tridentate-, binuclear- and polynuclear-complexes depending on the metal (Francis et al., 1992).
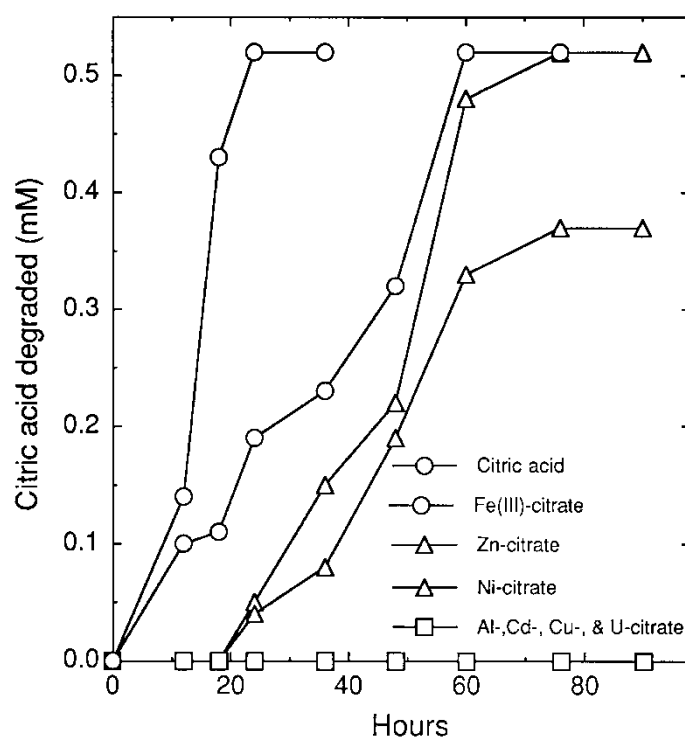
Figure 8. Biodegradation of metal citrate complexes by *Pseudomonas fluorescens*. Biodegradation is influenced by the type of complex formed between the metal and citric acid. Bidentate complexes (Ca-, Fe(II)-, Ni-, Zn-citrate) are readily degraded. Tridentate (Cd-, Cu-citrate), binuclear (U-citrate), and polynuclear (U-citrate) species are recalcitrant (Francis et al., 1992; Joshi-Tope and Francis, 1995).
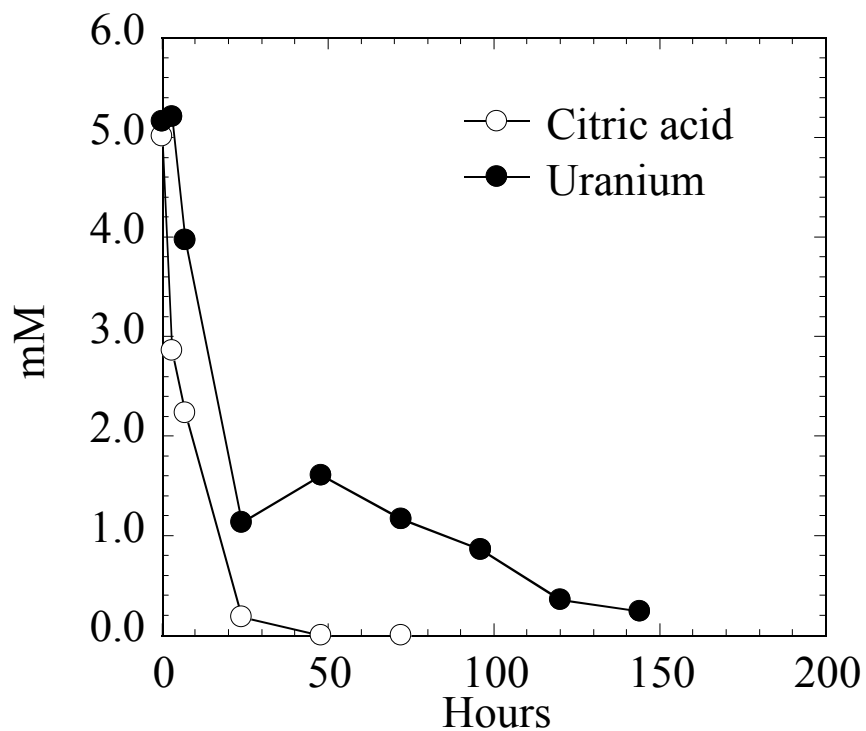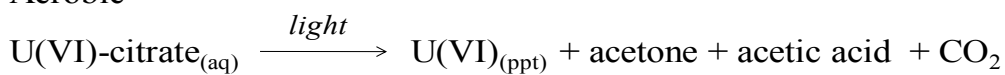
Figure 9. Photodegradation of the 1:1 U-citrate complex. Exposure of uranyl citrate complex to visible light (400- to 700-nm) showed photodegradation of citric acid to acetic acid and carbon dioxide, with precipitation of uranium as uranium trioxide ($UO_3 \cdot 2H_2O$) (Francis and Dodge, *unpublished results*)
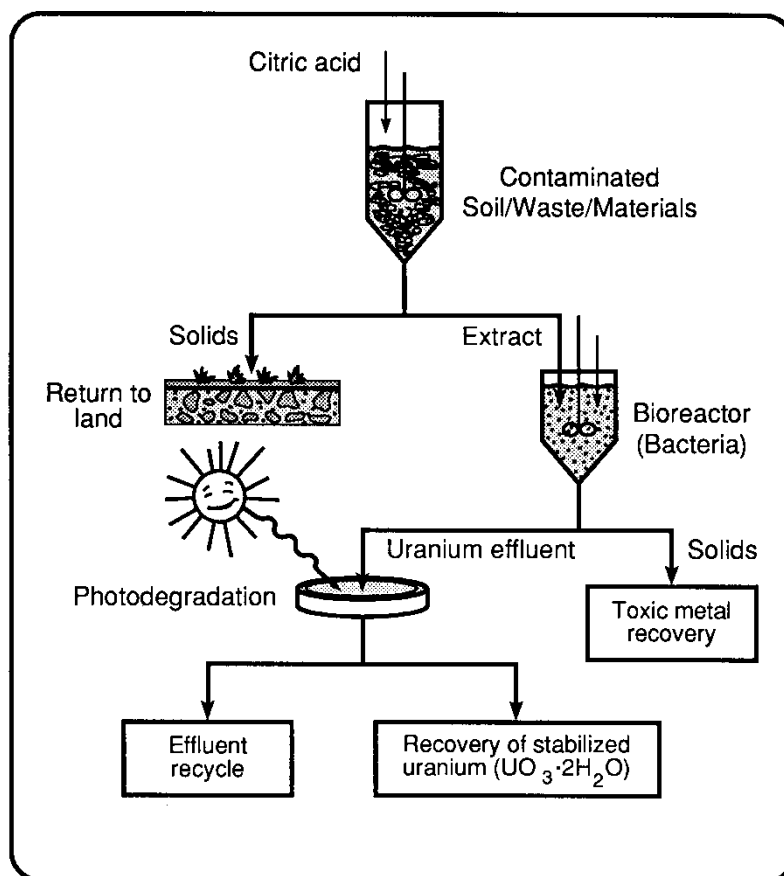
Figure 10. Citric acid process for treating uranium- and toxic metal-contaminated soils, sediments, and wastes (Francis and Dodge 1994; 1998).
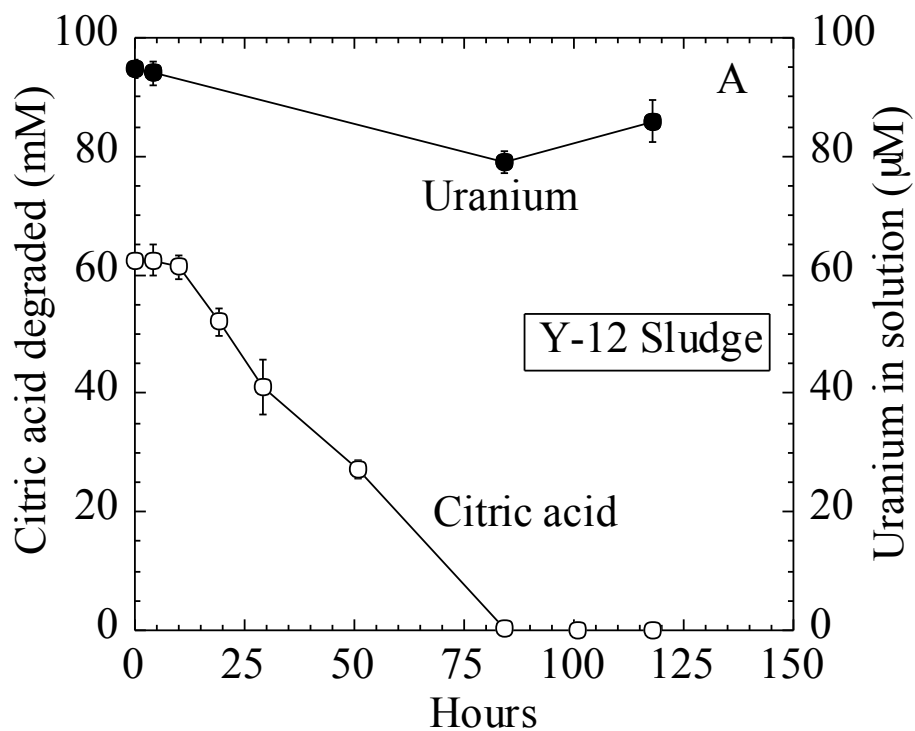
Figure 11A. Biodegradation of sludge citric acid extract (Francis and Dodge 1994; 1998).
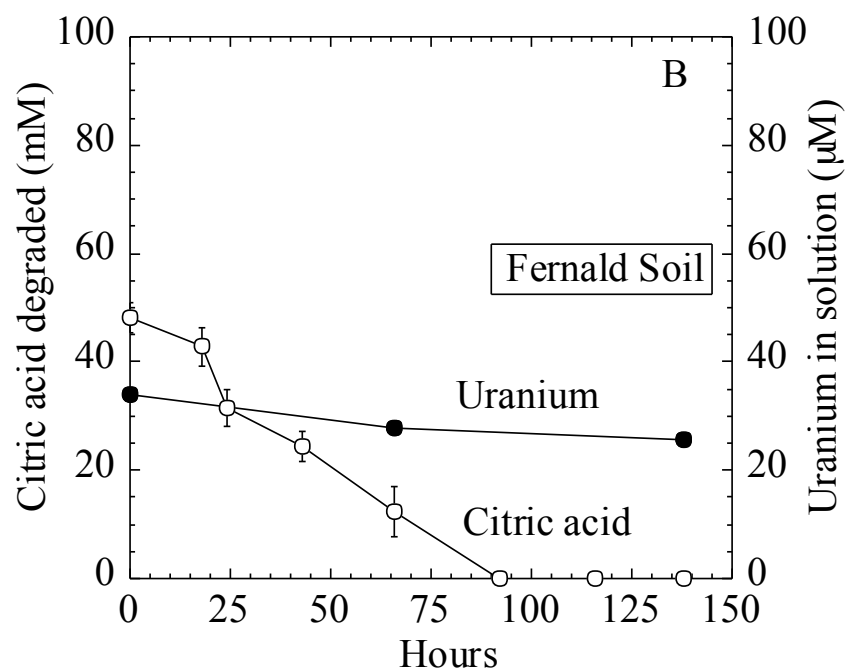
Figure 11B. Biodegradation of citric-acid extract of Fernald soil (Francis and Dodge 1994; 1998)
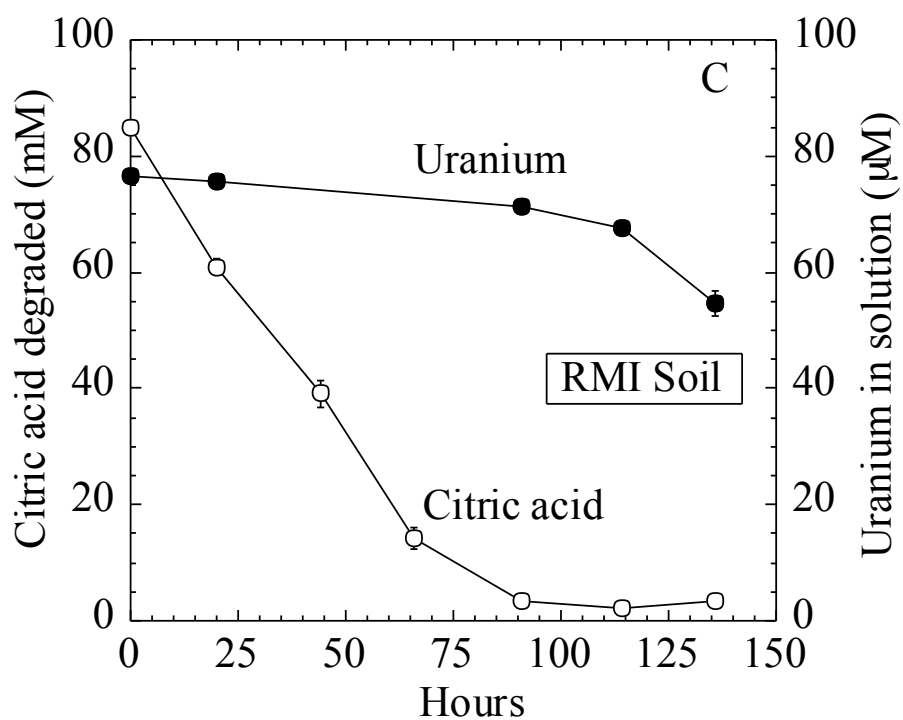
Figure 11C. Biodegradation of RMI Soil citric acid extract (Francis and Dodge 1994; 1998).
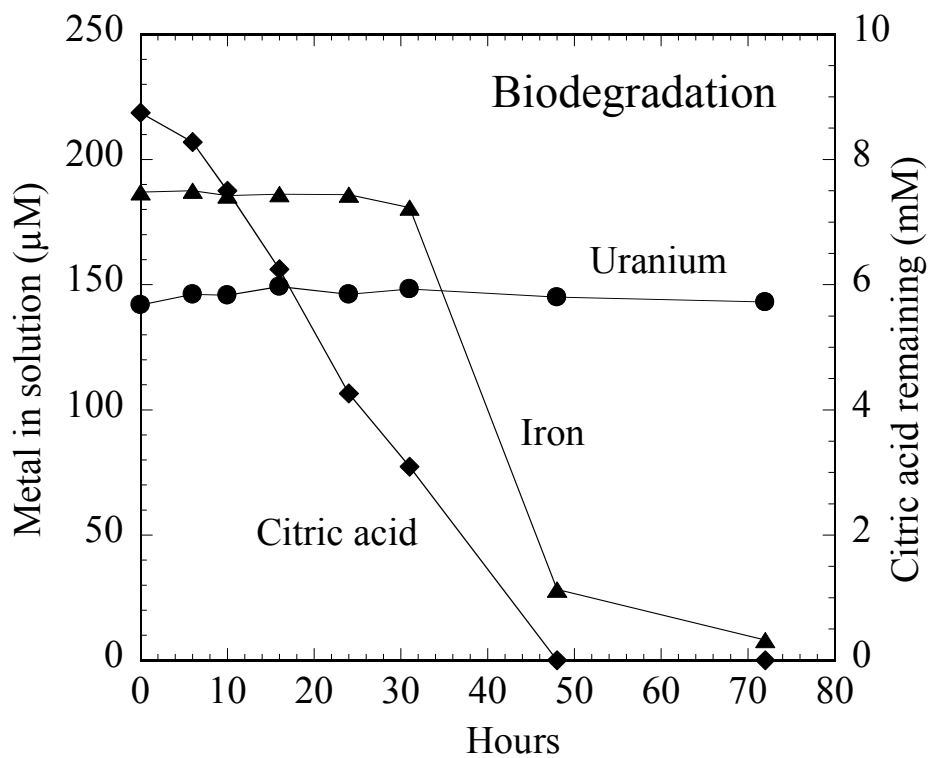
Figure 11D. Biodegradation of citric acid decontamination solution of uranium contaminated steel (Francis et al 2005)
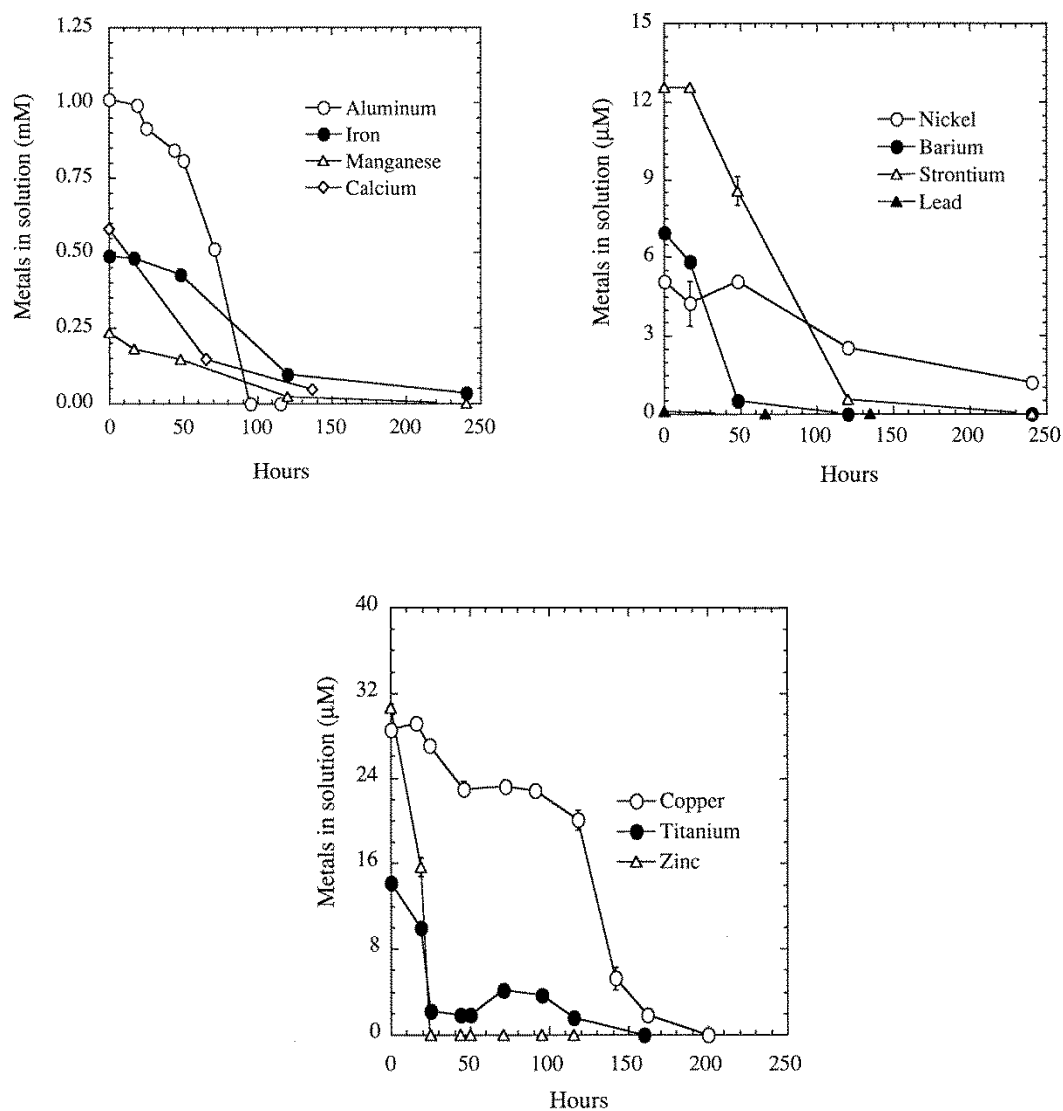
Figure 12. Fate of other metals during the biodegradation of the citric-acid extract from sludge (Francis and Dodge 1994; 1998).
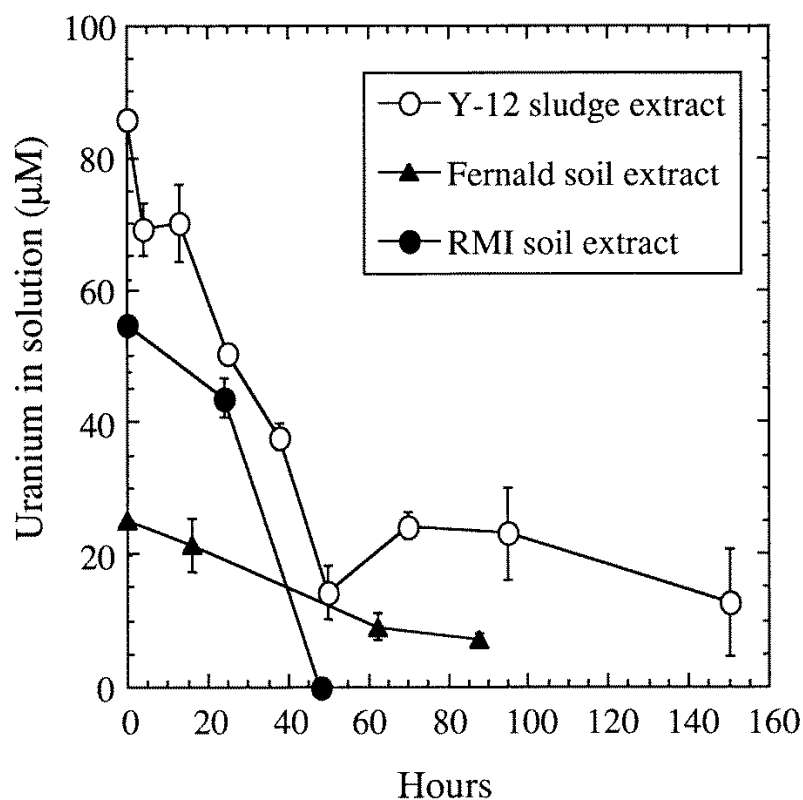
Figure 13. Photodegradation of uranyl citrate in citric-acid extracts after the biodegradation of citric acid and metal citrates (Francis and Dodge 1994; 1998).
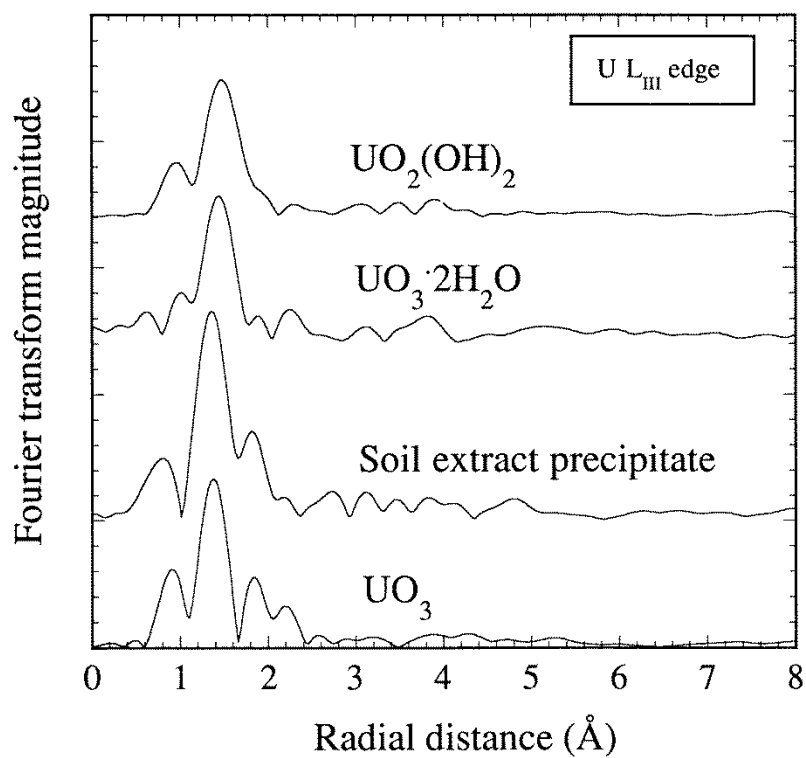
Figure 14. EXAFS spectrum of uranium precipitate (schoepite) recovered from the photodegradation of citric acid soil extract after biodegradation (Francis and Dodge 1998).
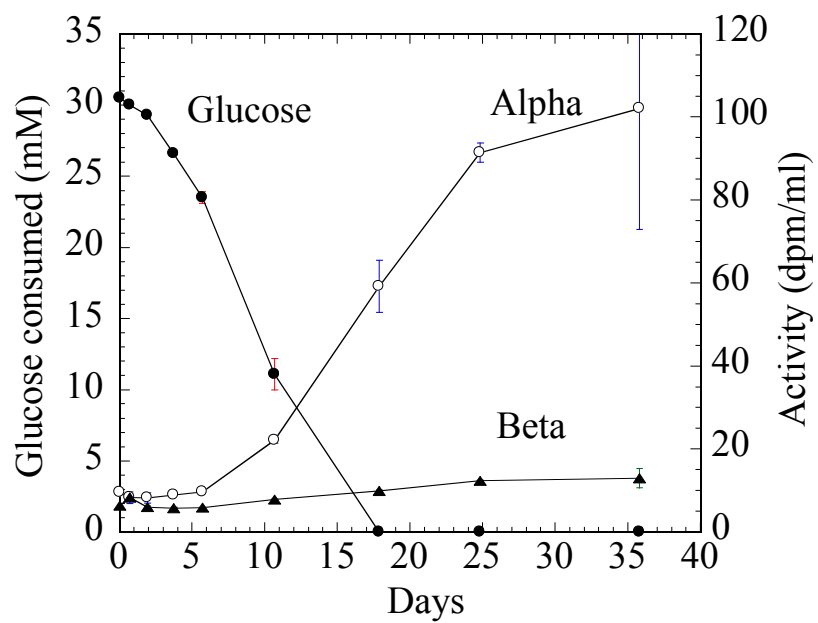
Figure 15. Mobilization of actinides due to microbial activity (Francis and Dodge, *unpublished results*).